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PhD thesis

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“Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less.”

Marie Curie

PREFACE AND ACKNOWLEDGEMENTS

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SUMMARY

Staphylococcus aureus (SA) is a ubiquitous bacterium in humans and animals. It can cause minor skin infections that do not usually require treatment. SA, as well as Methicillin-resistant SA (MRSA), which is resistant to a wide range of antimicrobials, can lead to severe infections in humans, especially in individuals with a suppressed immune status. MRSA can originate from livestock and is referred to as livestock-associated MRSA (LA-MRSA) with pigs as the main reservoir.

In Denmark, the number of pig herds tested positive for LA-MRSA has increased rapidly since 2008, when a Europe-wide study found only 3.5% of production herds positive for LA-MRSA. However, in 2016, the prevalence found in a national LA-MRSA screening had reached 88%. At the same time, the number of human cases increased in people working at pig farms but also in people with no livestock contact.

In 2014, Denmark initialised a national action plan to limit the spread of LA-MRSA. However, the effects were not sufficient to reduce the number of LA-MRSA-positive herds, nor to limit the number of newly infected humans. To be able to increase the efficacy of control programmes in the pig industry, it is necessary to understand the transmission routes of LA-MRSA among pig herds. This includes the trade of pigs as a potential mechanism for spread, as well as other contact among herds that might lead to the transmission of LA-MRSA. Such knowledge would help to evaluate control options that might reduce the number of LA-MRSA-positive herds in the future.

A network analysis using information on Danish pig holdings and their trade connections between 2006 and 2015 was performed to gain a general knowledge of temporal trends in the number of registered pig herds and pig movements. In addition, we investigated loyalty patterns and contact chains for the 24 registered holding types to identify holding types with a potentially higher risk of disease spread via pig movements (Manuscript I).

The total number of active holdings and the number of pig movements both decreased during the study period, while the holding size increased. We observed a large out-going contact chain for breeding and multiplier herds, which reflects the pyramidal structure of the underlying network and highlights the risk of spreading a disease via pig movements to a wide range of other herds if a pathogen were to affect breeding and multiplier herds. However, horizontal connections among pig herds also exist, which could lead to additional spread among herds of the same holding type.

An agent-based Monte Carlo simulation model mimicking the spread of LA-MRSA among pig herds was developed to study the epidemic behaviour and to identify the driving factors for LA-MRSA spread among pig herds (Manuscript II). As well as transmission based on pig movements registered in the study period from 2006 to 2015, indirect transmissions between holdings were modelled based on distance-dependent probability distributions. Three types of indirect contact were modelled: (1) abattoir trucks collecting pigs from several holdings, (2) humans such as veterinarians, farm workers or guests visiting several holdings

on the same day and (3) contact between herds with the same owner. Within-herd dynamics were modelled as a three-compartment SIS model with different transmission rates within the three compartments of sows, weaners and finishers, and with high- and low-risk transmission routes between these compartments. Several scenarios were evaluated to simulate the spread after introduction of LA-MRSA in varying proportions of breeding and multiplier and production herds in 2006 and/or 2009. We compared the model outcomes to the results of LA-MRSA surveys conducted in Denmark during the study period. An extensive sensitivity analysis was performed to study the effect of uncertainty of model parameters (Manuscript II).

Pig movements alone were not sufficient to mimic the observed increase in LA-MRSA-positive herds in Denmark in any of the modelled scenarios. However, the model identified three factors that played important roles in the between-herd spread of LA-MRSA: (1) the within-herd dynamics, (2) the frequency and effectiveness of indirect transmission via humans and (3) unexplained introduction of LA-MRSA to swine herds.

We enhanced this simulation model to retrospectively evaluate how different control strategies would have influenced the spread of LA-MRSA (Manuscript III). These strategies were combinations of the following control measures: (1) reduced numbers of herds using high-risk antibiotics, (2) reduced probability for indirect transmission between herds via humans, (3) movement restrictions and (4) voluntary eradication in 5-7.5% of the herds. The effects of implementing control in 2007 were compared to implementation in 2010.

Almost all tested control strategies simulated a reduction in the spread of LA-MRSA. The combination of two, three or four intervention strategies showed additive effects and led to larger reductions in the predicted herd prevalence. An extreme scenario (impeding the use of high-risk antibiotics, reducing risk of spread via indirect contact by 75%, implementing movement restriction and culling a percentage of positive herds) was able to reduce the predicted prevalence by 86% compared to the predicted prevalence without control. Control measures initialised in 2007 had a greater effect compared to initialisation in 2010 due to the lower initial prevalence in 2007.

In conclusion, pig movements alone were not sufficient to mimic the development of LA-MRSA herd prevalence observed in Denmark in 2008 and 2014. However, they were responsible for around 75% of transmissions. Within-herd dynamics and the frequency and effectiveness of indirect contact between pig herds via humans showed the highest impact on the predicted herd prevalence. Control measures showed the highest relative reduction when implemented in 2007 and when all four potential control measures were combined. Categorising herds according to the size of their out-going contact chain increased the effect of eradication as a control measure, as herds with higher risk of spreading LA-MRSA via pig movements were prioritised in the eradication process. However, eradication should be combined with movement restrictions to minimise the risk of re-introduction.

SAMMENDRAG (DANISH SUMMARY)

Staphylococcus aureus (SA) er almindeligt forekommende hos både dyr og mennesker. Denne bakterie kan forårsage mindre alvorlige hudsygdomme, der normalt ikke er behandlingskrævende. Methicillin-resistent SA (MRSA), der er resistente overfor en lang række antibiotika, kan forårsage alvorlige infektioner hos mennesker, særligt hos individer med nedsat immunforsvar. MRSA kan stamme fra husdyr, og denne gruppe af bakterier, der har primært reservoir hos svin, benævnes almindeligvis husdyr-MRSA.

I Danmark har der været en markant stigning i antallet af svinebesætninger, der er testet positive for husdyr-MRSA siden 2008, hvor der kun blev fundet husdyr-MRSA i 3,5 % af de danske besætninger, der blev undersøgt som del af en europæisk undersøgelse. I 2016, hvor der igen blev gennemført en dansk screening, var denne andel imidlertid steget til 88 %. I løbet af den samme periode steg forekomsten af humane tilfælde, ikke bare iblandt mennesker, der arbejder i svinebesætninger, men også blandt mennesker uden kontakt med husdyr.

I 2014, blev der i Danmark iværksat en handlingsplan, der havde til formål at mindske spredningen af husdyr-MRSA. Imidlertid var dette hverken tilstrækkeligt til at begrænse antallet af besætninger, der blev testet positive for husdyr-MRSA, eller til at begrænse antallet af nye humane tilfælde. For at kunne optimere effektiviteten af kontrolprogrammer i svineindustrien er det nødvendigt at opnå en bedre forståelse af, hvordan husdyr-MRSA overføres imellem svinebesætninger. Potentielle spredningsmekanismer, der kan resultere i spredning af husdyr-MRSA, inkluderer handel med svin såvel som anden kontakt imellem besætningerne. Denne viden vil være nyttig i forhold til at evaluere kontrolforanstaltninger, som potentielt kan være i stand til at reducere antallet af smittede besætninger.

I nærværende afhandling, er der udført en netværksanalyse baseret på information om danske svinebesætninger og deres handelspartnere i perioden 2006-2015 med henblik på at generere viden om variationen over tid i antallet af registrerede svinebesætninger og registrerede svineflytninger. Desuden blev stabiliteten af netværkene (loyalty patterns) og antallet af direkte og indirekte kontaktbesætninger (contact chains) undersøgt for 24 besætningstyper med henblik på at identificere besætningstyper med højere risiko for spredning af sygdom via svineflytninger (Manuskript I).

I løbet af studieperioden faldt antallet af aktive besætninger og antallet af svineflytninger, mens besætningsstørrelserne steg. Avls- og opformeringsbesætninger havde et højt niveau af direkte eller indirekte udgående kontakt til andre besætninger (outgoing contact chain), hvilket reflekterer pyramidestrukturen af det underliggende netværk og understreger risikoen for at sprede sygdom til en lang række andre besætninger via svineflytninger, såfremt avls- og opformeringsbesætninger inficeres med et patogen. Imidlertid eksisterer der også horisontale forbindelser imellem svinebesætningerne, hvilket kan lede til yderligere smittespredning imellem besætninger af samme type.

En individ-baseret Monte Carlo simuleringsmodel, der imiterer spredningen af husdyr-MRSA imellem besætninger blev udviklet med henblik på at studere, hvordan spredningen af MRSA udvikler sig og identificere de drivende faktorer bag spredningen imellem besætningerne (Manuscript II). Udover smitte baseret på svineflytninger registreret i studieperioden fra 2006-2015, blev indirekte spredning imellem besætninger modelleret baseret på afstandsafhængige sandsynlighedsfordelinger. Der blev modelleret tre typer af indirekte kontakter: 1) Slagtetransporter, hvor der afhentes svin fra flere forskellige besætninger, 2) Person-kontakt, f.eks. dyrlæger, landbrugsmedhjælpere eller gæster, der besøger flere besætninger i løbet af den samme dag, og 3) Ejer-kontakt mellem besætninger med samme ejer. Spredningsdynamikken indenfor samme besætning blev modelleret vha. en SIS-model med tre aldersgrupper og anvendelse af forskellige transmissionsrater indenfor hver af grupperne søer, fravænningsgrise og slagtesvin, og med hhv. høj- og lav risiko transmissionsruter imellem disse grupper. Adskillige scenarier blev simuleret med introduktion af husdyr-MRSA i variable andele af avl-, opformerings- og produktionsbesætninger i 2006 og/eller 2009, og spredningen mellem svinebesætninger blev evalueret. Modelresultaterne blev sammenlignet med resultaterne af screeningsundersøgelserne for husdyr-MRSA foretaget i Danmark i løbet af studieperioden og der blev udført omfattende sensitivitetsanalyser med henblik på at studere effekten af usikkerheden på model parametrene (Manuskript II).

Svineflytninger alene var ikke tilstrækkelige til at forklare den observerede stigning i antallet af husdyr-MRSA positive besætninger i nogen af de modellerede scenarier. Imidlertid identificerede modellen tre faktorer, som spiller en vigtig rolle i spredningen af husdyr-MRSA imellem besætninger: 1) Smittespredning indenfor besætningen, 2) Hyppigheden og effektiviteten af indirekte smittespredning via mennesker, og 3) Uforklarlig introduktion af husdyr-MRSA i svinebesætninger.

Vi udbyggede denne simuleringsmodel for retrospektivt at evaluere, hvordan forskellige kontrolstrategier ville have influeret spredningen af husdyr-MRSA (Manuskrift III). Disse strategier inkluderede kombinationer af følgende strategier: (1) En nedbringelse af antallet af besætninger, der anvender høj-risiko antibiotika, (2) Reduceret sandsynlighed for indirekte smittespredning imellem besætninger via mennesker, (3) Handelsrestriktioner, og (4) Frivillig sanering i 5-7,5 % af besætningerne. Effekten af at implementere kontrolforanstaltninger allerede i 2007 blev sammenlignet med implementering i 2010.

For næsten alle de undersøgte kontrolstrategier, resulterede simuleringerne i reduceret smittespredning. Kombinationer af to, tre eller fire interventionsstrategier havde en additiv effekt og ledte til større reduktion i de prædikterede prævalenser af positive besætninger. I et ekstremt scenarie, hvor brugen af høj-risiko antibiotika blev begrænset, risikoen for smittespredning via indirekte kontakt blev reduceret med 75 %, handelsrestriktioner blev implementeret og alle dyrene i en vis procentdel af de positive besætninger blev sendt til slagtning, blev den prædikterede prævalens af positive besætninger reduceret med 86 %, sammenlignet med den prævalens modellen prædikterede hvis ingen kontrolforanstaltninger var blevet iværksat. Som følge af den lavere prævalens i 2007 havde kontrolforanstaltninger igangsat i 2007 større effekt end kontrolforanstaltninger igangsat i 2010.

Det kan konkluderes, at svineflytninger ikke alene kan forklare udviklingen i andelen af MRSA-positive besætninger i Danmark i perioden 2008-2014. Imidlertid var svineflytninger ansvarlige for ca. 75 % af smittespredningen. Spredningsdynamikken indenfor besætningen og hyppigheden og effekten af indirekte kontakt imellem svinebesætninger via mennesker havde den største indflydelse på de prædikterede prævalenser af positive besætninger. Kontrolforanstaltninger resulterede i den største relative reduktion, når de blev implementeret i 2007, mens prævalensen af positive besætninger endnu var lav, og når alle fire potentielle kontrolstrategier blev kombineret.

Effekten af sanering som kontrol foranstaltning øges, hvis besætninger med højere risiko for at sprede husdyr-MRSA via svineflytninger blev prioriteret mht. sanering. Sanering bør imidlertid kombineres med flytterestriktioner for at minimere risikoen for re-introduktion.

ZUSAMMENFASSUNG (GERMAN SUMMARY)

Staphylococcus aureus (SA) ist ein ubiquitäres Bakterium, welches auf der Haut und Schleimhaut von Menschen und Tieren vorkommt. Es kann leichte Hautentzündungen hervorrufen, die in der Regel keine Behandlung benötigen. Nutztier-assoziierte Methicillin-restistente SA (LA-MRSA) sind gegen alle bisher verfügbaren β-Lactam-Antibiotika (z. B. Penicillin), aber auch gegenüber anderen Antibiotikaklassen resistent. Sie können deshalb, vor allem bei Menschen mit geschwächtem Immunsystem, schwere Infektionen hervorrufen. Hauptreservoir von LA-MRSA sind Schweine.

In Dänemark ist die Anzahl an Schweinebetrieben, die positive auf LA-MRSA getestet wurden seit 2008 rapide angestiegen. In einer Europaweiten Studie wurden damals nur 3,5 % der Schweinemastbetriebe positiv getestet. Im Jahre 2016 lag die Prävalenz in einer nationalen Studie jedoch bereits bei 88 %. Gleichzeitig ist die Anzahl an gemeldeten Fällen bei Menschen mit, aber auch ohne Kontakt zu Schweinebetrieben stark angestiegen.

Dänemark beschloss 2014 einen nationalen Aktionsplan, um die Ausbreitung von LA-MRSA einzudämmen. Die Maßnahmen reichten jedoch weder dazu aus, die Anzahl an positiven Beständen zu reduzieren, noch die Anzahl an Neuinfektionen beim Menschen zu begrenzen. Um die Wirksamkeit von Bekämpfungsmaßnahmen in der Schweineindustrie zu erhöhen, ist ein besseres Verständnis der Übertragungswege von LA-MRSA zwischen den Schweinebetrieben nötig. Dies umfasst den Schweinehandel als möglichen Übertragungsweg genauso wie andere Kontakte zwischen den Herden, die zur Übertragung führen können. Ein solches Wissen würde die Beurteilung von potentiellen Bekämpfungsmaßnahmen ermöglichen, um in Zukunft die Anzahl an LA-MRSA positiven Beständen zu verringern.

Als erstes wurde in dieser Arbeit eine Netzwerkanalyse durchgeführt, um allgemeine Informationen über die Entwicklung der Bestandszahlen und Handelskontakte zu erhalten. Grundlagen für diese Analyse waren Daten über dänische Schweinebetriebe und ihrer Handelsbeziehungen zwischen 2006 und 2015. Zusätzlich wurde die Loyalität der Handelsbeziehungen und die Länge von Handelsketten für die 24 registrierten Bestandstypen untersucht. Ziel war es, Bestandstypen mit potentiell höherem Risiko für die Verbreitung von Krankheiten über den Schweinehandel zu identifizieren (Manuskript I).

Die Anzahl an aktiven Beständen und registrierten Handelskontakten sank in dem betrachteten Zeitraum, während die Bestandsgrößen anstiegen. Die Handelsketten von Zuchtbetrieben waren im Vergleich zu anderen Bestandstypen deutlich länger, und spiegeln damit die Pyramidenstruktur des zugrunde liegenden Netzwerkes wider, an dessen Spitze sich die Zuchtbetriebe befinden. Außerdem hebt dies die Bedeutung hervor, die der Eintrag eines Erregers in Zuchtbetriebe auf das Risiko der Ausbreitung im Handelsnetz haben kann. Neben vertikalen Verbindungen wurden jedoch auch horizontale

Handelsverbindungen zwischen Betrieben des gleichen Typs beobachtet, die zu einer weiteren Ausbreitung von Erregern führen kann.

Im zweiten Teil dieser Arbeit wurde ein agentenbasiertes Monte Carlo Simulationsmodell entwickelt, welches die Ausbreitung von LA-MRSA zwischen den Schweinebetrieben in Dänemark nachbildet. Ziel war es, das epidemische Verhalten von LA-MRSA zu untersuchen und die Hauptwege der Ausbreitung zwischen den Schweinebeständen zu identifizieren. Neben der Übertragung auf dem Handelsweg basierend auf registrierten Handelsbewegungen in dem Studienzeitraum von 2006 bis 2015, wurden auch indirekte Kontakte zwischen den Betrieben modelliert. Diese basierten auf distanzabhängigen Verteilungen und folgende Übertragungswege wurden betrachtet: (1) Lastwagen, die Schweine von mehreren Betrieben zum Schlachthof transportieren, (2) Menschen (z.B. Veterinäre, Farmarbeiter oder Gäste), die mehrere Betriebe am gleichen Tag aufsuchen und (3) Kontakte zwischen verschiedenen Herden mit dem gleichen Besitzer. Die Ausbreitung innerhalb der Bestände wurde als SIS-Model implementiert und umfasste die Übertragung mit verschiedenen Übertragungsraten innerhalb und zwischen den drei Bereichen für Sauen, Ferkel und Mastschweine. Die Anzahl der Tiere je Bereich in den zwei Gruppen: (S) Empfängliche für LA-MRSA, und (I) positiv für LA-MRSA wurden dabei täglich aktualisiert. Verschiedene Szenarien, die die Ausbreitung nach dem Eintrag von LA-MRSA in unterschiedliche Anteile der Zucht- und/oder Mastbetriebe nachstellen, wurden miteinander verglichen. Die modellierten Ergebnisse wurden mit LA-MRSA Studienergebnissen von 2008 und 2014 verglichen. Eine ausführliche Sensitivitätsanalyse untersuchte den Einfluss der Modellparameter auf die Modellergebnisse (Manuskript II).

Der Tierhandel allein war in keinem der simulierten Szenarien ausreichend, um den Anstieg an LA-MRSA positiven Herden in Dänemark zu erklären. Das Modell identifizierte jedoch drei Faktoren, die eine wichtige Rolle bei der Ausbreitung von LA-MRSA zwischen Schweinebeständen spielten: (1) die Prävalenz in den Herden, (2) die Häufigkeit und Effektivität von indirekten Kontakten zwischen den Beständen durch Menschen und (3) unerklärliche Einträge von LA-MRSA in Schweinebestände.

Das Model wurde im dritten Teil dieser Arbeit erweitert, um zu untersuchen, welchen Effekt verschiedene Bekämpfungsmaßnahmen auf die Ausbreitung von LA-MRSA gehabt hätten, wenn sie bereits während des Studienzeitraumes angewendet worden wären. Die folgenden Maßnahmen und deren Kombinationen wurden implementiert: (1) Reduzierung der Anzahl an Beständen, die Risiko-Antibiotika verwenden, (2) Reduzierung der Wahrscheinlichkeit für die indirekte Übertragung von LA-MRSA zwischen Beständen durch Menschen, (3) Handelsbeschränkungen, die den Handel von positiv getesteten Herden zu negativen Beständen verbieten und (4) freiwillige Bestandssanierung in 5-7,5 % der Herden. Die Auswirkungen dieser Bekämpfungsmaßnahmen unter der Annahme, dass diese bereits in 2007 oder 2010 eingeführt wurden, wurden untersucht und verglichen (Manuskript III).

Fast alle untersuchten Bekämpfungsmaßnahmen und -strategien reduzierten die Ausbreitung von LA-MRSA in den sechs Jahren nach Einführung der Maßnahmen. Die Kombination von zwei, drei oder allen vier Maßnahmen zeigte einen additiven Effekt und führte zu einer höheren Reduzierung der modellierten Herdenprävalenzen. Die drastische Kombination aus dem Verbot der Nutzung von Risiko-Antibiotika, der Reduktion der Übertragung durch indirekte menschliche Kontakte um 75 %, Handelsbeschränkungen und Bestandssanierungen führte zu einer Reduktion der Herdenprävalenz um 86 % verglichen mit dem Szenario ohne Bekämpfungsmaßnahmen. Die Einführung der Maßnahmen in 2007 führte durch die niedrigere Prävalenz in 2007, zu stärkeren Effekten verglichen mit dem Start der Bekämpfung in 2010.

Zusammenfassend kann festgestellt werden, dass Handelskontakte nicht ausreichend waren, um die Entwicklung von LA-MRSA, wie sie in Dänemark beobachtet wurde, zu erklären. Trotzdem war der Handel in dem vorgestellten Simulationsmodell in ungefähr 75 % der Fälle verantwortlich für die Übertragung. Die Prävalenz innerhalb der Herden und die Häufigkeit und Effektivität der Übertragung zwischen Beständen durch den Menschen zeigte den größten Einfluss auf die Ausbreitung von LA-MRSA zwischen den Beständen. Bekämpfungsmaßnahmen zeigten das höchste Potential zur Reduktion der Ausbreitung, wenn sie in dem Modell im Jahr 2007 eingeführt und wenn alle vier Maßnahmen kombiniert wurden. Wenn für die Auswahl der Bestände, die eine Sanierung durchführen, die Länge der Handelsketten zu Grunde gelegt wurde und damit Herden ausgewählt wurden, die ein größeres Potential zur Verbreitung von LA-MRSA über das Handelsnetz zeigten, erhöhte dies den Effekt der Sanierungsmaßnahmen. Allerdings sollte die Sanierung von Beständen mit Handelsrestriktionen kombiniert werden, um das Risiko des Wiedereintrags zu minimieren.

List of Abbreviations

CHR	Central Husbandry Register
GSCC	Giant strongly connected component
LA-MRSA	Livestock-associated Methicillin-resistant *Staphylococcus aureus*
MRSA	Methicillin-resistant *Staphylococcus aureus*
PMD	Pig movement database
SA	*Staphylococcus aureus*
SPF	Specific pathogen-free

LIST OF ORIGINAL PUBLICATIONS

Schulz J, Boklund A, Halasa T, Toft N and Lentz H (2017). Network analysis of pig movements: Loyalty patterns and contact chains of different holding types in Denmark. *PLoS ONE* **12** (6): e0179915. https://doi.org/10.1371/journal.pone.0179915

Schulz J, Boklund A, Toft N and Halasa T (Accepted at *Scientific Reports*). Drivers for livestock-associated Methicillin-resistant Staphylococcus aureus spread among Danish pig herd – a simulation study.

Schulz J, Boklund A, Toft N and Halasa T (Submitted to *Scientific Reports*). Effects of control measures on the spread of LA-MRSA among Danish pig herds between 2006 and 2015 – a simulation study

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1. INTRODUCTION

1.1. MOTIVATION

Disease spread models are widely used in the field of veterinary medicine[1,2]. They allow for a better understanding of the driving mechanisms behind pathogen spread within or between livestock farms. Additionally, potential control options could be simulated to evaluate the effectiveness of interventions and thus help decision makers set up meaningful control programmes.

Livestock-associated Methicillin-resistant *Staphylococcus Aureus* (LA-MRSA) is a pathogen that mainly occurs in pigs but has also been found globally in other species like cattle, horses, rodents, companion animals and humans[3–10]. A survey conducted by the European Commission in 2008 found 3% of Danish pig production herds were positive for LA-MRSA. Since then, there has been a rapid increase in the number of LA-MRSA-positive herds, with 88% of production herds found positive for LA-MRSA in 2016.

During the same period, there has been a marked increase in the number of human LA-MRSA cases in Denmark[11]. Some of these cases could be explained by the revised MRSA guidelines, which aims to test more people at high risk. Nevertheless, since LA-MRSA can be transmitted between pigs and humans, there is a need to control LA-MRSA in the pig industry to limit its spread to humans.

There is a lack of knowledge about how LA-MRSA spreads among pig herds. The importance of the different transmission routes, including pig movements and indirect contact, is unknown. Gaining better insight into pig movements, and therefore into the structure of the Danish pig industry, would aid in the development of a simulation model of LA-MRSA spread among Danish pig herds. Such a model would provide a chance to understand and assess the impact of LA-MRSA transmission routes and to investigate potential intervention strategies. It could therefore help decision makers enhance the implemented control strategy.

1.2. Outline and objectives

The primary objective of this thesis was to gain insight into influential transmission mechanisms of LA-MRSA among pig herds and to investigate the effects of potential control strategies against this transmission. More precisely, the aims were:

1. To identify patterns of contact between pig herds and potential hubs for disease spread.
2. To understand and assess the impact of potential routes of LA-MRSA transmission among Danish pig herds.
3. To investigate the effect of different control strategies on the spread of LA-MRSA among Danish pig herds.

This thesis begins with a short introduction to the pig industry in Denmark, as well as an overview of the occurrence, spread and control of LA-MRSA in Danish pig herds (Chapter 1). Chapter 2 gives an overview on the materials and methods used. The results were summarised in the three manuscripts presented in Chapter 3. Network analysis tools were used to gain a better insight into the contact patterns among pig herds and their loyalty to trade partners. Additionally, the size of a herd's out-going contact chain was investigated as a measure of their risk of spreading a disease via pig movements, and subsequently their potential to act as hubs for disease spread (Manuscript I). Furthermore, a simulation model was developed to mimic LA-MRSA spread (Manuscript II) and the effects of potential control measures on LA-MRSA spread among Danish pig herds (Manuscript III). Chapter 4 presents the most important results from the manuscripts and this thesis, followed by a general discussion in Chapter 5 highlighting the interconnectivity of the presented studies and an evaluation of the potential consequences of gaps in our knowledge on the results. Conclusions (Chapter 6) and perspectives (Chapter 7) complete this thesis.

1.3. Pig production in Denmark

Denmark is one of the ten largest pig-producing countries worldwide[12]. The export of pork constitutes about 5% of Danish exports, making Denmark one of the largest pig meat exporters[12].

Information on pig holdings is gathered in the Central Husbandry Register (CHR), which is owned by the Danish Ministry of Environment and Food. For all Danish pig holdings, the CHR holds information on the identification number of the holding and enterprise, the holding type, and the number of sows, finishers, and weaners in each holding. The owner must indicate the type of holding during the registration process and is responsible for keeping the information in the CHR updated. Furthermore, at least once or twice a year depending on herd size, a letter is distributed to each herd owner inviting them to confirm or correct their herd information in the CHR, including the number of animals. If a herd is closed, the closure must be reported no more than 6 months after the last animal has left the herd. (https://www.retsinformation.dk/Forms/R0710.aspx?id=185121).

Danish pig production has a pyramidal structure[13,14]. Breeding-related holdings (breeding and multiplier herds, quarantine stations and boar stations) form the top of the pyramid and sell breeding stock. In the centre, production sites (production herds, weaner herds, free-range pig herds, organic herds) produce weaners and/or finishers for sale. At the bottom of the pyramid, end-of-production sites (slaughterhouses, export isolation facilities, collection points for dead animals, cooling stations, rendering plants) complete the production process. Transit sites (traders, trade herds, pig shows, livestock auctions, collection points, slaughter animal markets) support the trade among the pig herds. Most trade connections are vertical – from the top to the bottom of the production pyramid. Nevertheless, horizontal connections between pig herds also exist. Besides these central holding types, pig owners can also register hobby sites (hobby herds, pets, wild boar herds, organic wild boar herds) and miscellaneous herds (zoos, experimental facilities).

No definitions of the 24 pre-defined holding types are available in the CHR. There is one category covering "breeding and multiplier herds", even though breeding herds produce and sell purebred breeding stock, while multiplier herds produce and sell hybrids. Additionally, the category "breeding and multiplier herds" does not necessarily only cover herds that registered sows for breeding, but could include any kind of holding due to self-registration by the owner. In general, this is true for all holding types.

The CHR contains information on pig movements, including identification numbers for the holding and the enterprise for both sending and receiving herds. Among other things, the number of moved pigs and the date of the pig movement are registered.

To ensure a high level of quality, a closed production and health system (SPF – specific pathogen-free) has been established in Denmark[12]. Farms enrolled in the SPF system are organised depending on their health status. This includes the safety level, health declarations and additional information on the health status. In 2018, 3,100 herds in Denmark were enrolled in the SPF system, and were therefore obliged to follow the rules of SPF (www.spf-sus.dk/en, visited on 13th August 2018). Furthermore, an unknown number of herds not enrolled in the SPF system more or less follow the same set of biosecurity rules, but are not tested regularly for diseases included in the SPF system[15].

1.4. Livestock-associated methicillin-resistant *Staphylococcus aureus*

Staphylococcus aureus (SA) is an opportunistic pathogen in humans and animals. It does not usually cause clinical signs, in which case, the humans or animals are termed 'colonised'. In the case of a reduced immune system, SA can spread and cause minor skin infections that do not usually require treatment. Under unfavourable conditions, it can lead to severe infections such as pneumonia or sepsis that could result in death. If SA gains resistance against antibiotics, treatment becomes difficult. Methicillin-resistant SA is a group of SA that is resistant to beta-lactam antibiotics. MRSA is often multi-resistant, with additional resistance against other antimicrobials such as tetracycline. Livestock-associated MRSA (LA-

MRSA) was first described in the Netherlands in 2005, and a special clonal complex, CC398, was identified shortly after[16]. LA-MRSA has been found in several livestock animals such as pigs, cattle and poultry[16–18]. However, LA-MRSA also colonises humans who take care of the livestock[16,19–21]. The number of human cases without livestock contact has increased in recent years[11], and as such, there are indications for human-to-human transmission[22].

1.4.1. Occurrence of LA-MRSA in the pig population

Since its first occurrence in the Netherlands and France in 2005, LA-MRSA has been reported worldwide: in Europe, a baseline study conducted in 2008 found LA-MRSA in 19 of 27 European countries[3]. Later, LA-MRSA CC398 occurrence was reported in Asia[4,23], North America[10,24,25], Northern Africa[5], South America[26] and Australia[27]. Besides CC398, other clonal complexes are also circulating in pig populations[4,28,29]. However, the focus in this thesis is on CC398, and only this strain is simulated in the model described.

In Denmark, several LA-MRSA screenings have been performed since 2008, when 3.5% of Danish pig production herds were found positive for LA-MRSA in the European baseline study. In the European baseline study, five dust samples were taken in the immediate environment of breeding pigs in the holdings and then pooled and tested for LA-RMSA[3]. Environmental swabs were reported as an easy-to-use and non-invasive method. Nevertheless, the number of samples is dependent on the within-herd prevalence[31] and there is a risk that herds with a low prevalence will not be detected when using this method. However, Broens et al.[31] recommend avoiding pooling of environmental samples, instead investigating them individually to increase the sensitivity, especially in herds with a low within-herd prevalence. The results of the EU baseline study might therefore be an underestimation.

Three screenings for LA-MRSA at slaughterhouses were performed in Denmark between 2009 and 2012[32–34]. During that period, the observed prevalence increased from 13% of pigs testing positive at slaughter in 2009 to 77% in 2012. When interpreting these results, it is important to take into account that LA-MRSA might spread during transportation to the slaughterhouse and in the slaughterline[35]. The results might therefore overestimate the occurrence.

In 2014, a national survey found 63% of breeding herds and 68% of production herds positive for LA-MRSA[36] by testing pools of five nasal swabs per herd. In 2016, 88% of Danish pig production herds were tested positive for LA-MRSA[11].

1.4.2. Risk factors for the occurrence and prevalence of LA-MRSA in a pig herd

Even though this thesis focuses on the between-herd spread of LA-MRSA, the presence and prevalence of LA-MRSA within pig herds will influence its spread between herds. Therefore, risk factors for the occurrence of LA-MRSA in a pig herd are relevant in setting up meaningful control programmes to also limit the spread between pig herds.

On rare occasions, LA-MRSA could spontaneously emerge in a pig herd[37]. Nevertheless, the main reason for the occurrence of LA-MRSA in a pig herd is introduction via external sources. Herd characteristics and herd management strategies seem to influence whether or not LA-MRSA could spread in a pig herd after introduction[38–41].

Alt et al.[39] found large (> 1,000 finishers) and medium-sized (500-999 finishers) herds had a higher risk of LA-MRSA occurrence. The production type was also associated with the presence or absence of LA-MRSA[39,41]. In a study performed in the Netherlands, the herd type was also found to be a risk factor: the herd prevalence of LA-MRSA in organic pig herds was reported to be lower compared to conventional pig herds[42]. In Germany, none of the investigated organic pig herds tested positive for LA-MRSA in 2012[43].

The number of sources from which a pig herd purchased animals was significantly associated with the occurrence of LA-MRSA in pig herds in a univariate analysis[39,41].

Low levels of antibiotics in feed promote improved growth and health benefits in pigs[44,45]. Nevertheless, group treatment with antimicrobials has been identified as risk factor for the presence of LA-MRSA in some studies[38,39] and led to higher transmission rates and therefore a higher prevalence within the tested pig herds[46]. LA-MRSA could also be resistant to zinc, which is frequently used for the treatment of diarrhoea in weaning pigs. The use of zinc could increase the prevalence in weaners and thus lead to the persistence of LA-MRSA in pig herds[47].

1.4.3. Risk factors for the spread of LA-MRSA among pig herds

Pig movements were described as an important route for the spread of LA-MRSA among pig herds[35,48,49]. Nevertheless, there are examples of herds that did not receive pigs from other herds, yet were still affected by LA-MRSA[50]. Other routes of transmission must therefore exist.

People working at pig farms (farmers, veterinarians, etc.) have been reported to have a higher risk of being colonised with LA-MRSA[51,52]. Despite this, reports of human-to-pig transmission are rare, and it is assumed that LA-MRSA was introduced to three pig herds in Norway by humans[53]. A German study in LA-MRSA-positive herds found 86% of the farmworkers and 45% of the associated veterinarians were positive for LA-MRSA[54]. A Dutch study showed that veterinarians carried the same LA-MRSA strain for up to 14 months, though it remains unclear whether the LA-MRSA was persistent or reacquired[55]. The veterinarians in this study visited two to three different farms each working day and up to 10 different farms each week[55].

Short-term visitors on pig farms seem to carry LA-MRSA mainly transiently, and most visitors tested negative for LA-MRSA after 24 hours[56]. However, there is still a minor risk of spreading LA-MRSA among pig herds when visits are within a short time period.

Other livestock, companion animals, rodents and flies were reported to carry LA-MRSA[38,39,50,57,58]. Their role in the transmission between pig herds is not quantified, but it has been suggested that transmission could occur[50].

A discussion on whether LA-MRSA could be transmitted via the environment is still ongoing. LA-MRSA has frequently been detected in dust samples[17,30,59,60]. In addition, colonisation via air was recently proven in an animal experiment[61]. Nevertheless, there is no evidence that herd-to-herd transmission via air is possible. LA-MRSA has been found in soil close to pig herds in Germany[62,63], as well as in slurry[63].

1.4.4. Prevention and control of LA-MRSA spread within and between pig herds

Prevention and control strategies for LA-MRSA are mainly based on known risk factors for its occurrence in and spread between pig herds.

To prevent the spread of LA-MRSA within a pig herd, Catry et al.[64] recommend reducing the use of antibiotics as prophylactic treatment and group treatment in order to reduce the selective pressure. Besides the potential reduction in within-herd transmission rates[46], an additional benefit would be to preserve the efficacy of current antimicrobials – both in the veterinary field, and for humans.

As pig movements play an important role in the spread of LA-MRSA among pig herds[48], movement restrictions on trade from LA-MRSA-positive to negative herds could also be considered as a prevention strategy[64]. Taking into account the pyramidal structure of Danish pig production, eradication of LA-MRSA in breeding herds could be prioritised in order to minimise transmission to herds further down the production line[64]. In countries with low herd prevalence, eradication could be an option[53]. However, LA-MRSA is endemic in Denmark and eradication (i.e. sending all pigs from an LA-MRSA-positive herd to slaughter, followed by intense cleaning and disinfection of the farm and restocking with LA-MRSA-negative pigs) would be an extremely costly[65] and impractical scenario. It is not realistic that such a strategy would be acceptable for the swine industry.

Cleaning and disinfection of farms has been proven to remove LA-MRSA in the absence of animals[66]. Nevertheless, MRSA showed resistance against quaternary ammonium compounds, a component of disinfectants[67], which might pose a challenge to successful disinfection.

In 2014, the first actions against LA-MRSA were established in Denmark, including:

- hygiene measures to limit the risk of bacteria being carried out of the herd
- an infection protection plan issued by the farmer and the herd's veterinarian
- discontinuation of routine group medication of pig herds
- establishment of an advisory service for pig and health workers
- further investigations on antibiotic usage regulations and on alternative vaccination[68]

A year later, a four-year action plan was established by the Danish Ministry of Food and Environment based on the recommendations of an expert group[50,68]. This action plan covered:

- a 15% reduction in the use of antimicrobials for pigs
- hygiene measures focusing on the prevention of LA-MRSA spread into the community and the working environment in pig herds
- reduction of contamination in the herds
- surveillance of the LA-MRSA prevalence
- investigation of the transmission routes of LA-MRSA
- international efforts to promote a joint EU strategy to reduce antimicrobial resistance.

Nevertheless, the success of this action plan in controlling LA-MRSA between Danish pig herds is currently unknown.

1.5. Network analysis

Animal trade plays an important role in the spread of LA-MRSA among pig holdings[48]. Describing pig movements might help us to gain a better insight into the nature and extent of contact between pig herds, and subsequently the potential risk for LA-MRSA transmission among herds. The characterisation of herds within the network and the identification of important components of the network might provide important information for decision makers when developing control programmes[69].

The first published studies to use network analysis tools were conducted in the field of human psychology[70], although the concepts might have been developed earlier[71]. In the veterinary field, network analysis has been used since 2003[72,73]. In early publications, static snapshots of the underlying networks were investigated, in which the time of the connection between two elements of the network was ignored. However, the time at which a trade connection between two holdings occurs is important for disease spread, and dynamic network analysis tools have recently been used in the veterinary field[74].

1.6. Simulation modelling

A simulation model attempts to represent physical, chemical or biological processes using computer programs developed to mimic simplified versions of these processes in order to explore how a situation may develop in response to different interventions. Computer-assisted modelling and simulation of epidemics have gained importance in veterinary medicine in recent years[1]. In many cases, building a simulation model will offer an insight into the patterns of disease spread and enable a better understanding of the interaction of different influential factors for disease spread and persistence. Additionally, models could assist in deriving strategies for the control of endemic and epidemic diseases.

In the veterinary field, simulation models cover a wide range of applications such as modelling the within-host infection dynamics, disease-spread modelling within or between populations and evaluating control strategies to support decision makers in the control of infectious diseases[1].

Simulation modelling provides the opportunity to gain a better insight into complex processes, especially in cases where experimental studies are not possible[1,2]. However, simulation models typically involve parameters that are either unknown or only roughly estimated. Together with model assumptions made to simplify the real-life process, this could lead to uncertainties in the model outcome. The ethical good practice guidelines described highlight the importance of clearly communicating model results, limitations and uncertainties in order to avoid misinterpretation and help make the best possible decisions[75,76].

In addition to the individual-based (or agent-based) modelling approach used in this thesis, population-based models have also been described[2,77]. Population-based models simulate groups of individuals, for example susceptible or infected individuals. The number of groups is based on the modelled disease, and might be related to the disease status, age or gender classification[77]. In contrast, individual-based models mimic the status of each individual over the simulated time period and simulate the actions and interactions of autonomous agents (either individual or collective entities), with a view to assessing their effects on the modelled system as a whole.

Population-based models do not usually allow complex control programmes to be mimicked mechanistically, as only proportions of individuals are modelled and thus the assumption is necessary that the intervention is applied for all individuals in a certain group, which might not be realistic.

Individual-based models allow for the mechanistic evaluation of control measures, i.e. based on an understanding of the processes within the studied system. These models are usually based on data from recent outbreaks or the literature, expert options on the underlying processes, as well as estimates for unknown parameters. Model validation and sensitivity analysis can help us to gain a better understanding of the uncertainties resulting from the assumptions made and information used to build the model.

1.6.1. Simulation modelling of LA-MRSA

In addition to the between-herd spread, simulation modelling related to LA-MRSA has been described in the following areas: within-herd spread[78,79], spread along the pig slaughter line[80], and transmission from pigs to humans[81].

To date, two between-herd models have been described in the literature: Ciccolini et al.[82] used Danish movement data from two separate years to simulate the spread of LA-MRSA among Danish pig herds. Within-herd dynamics were ignored in the model, as it was assumed that once a herd was affected by LA-MRSA, a certain proportion of pigs would rapidly become LA-MRSA positive. No control measures were included in that model. This study found a significant probability of long-term LA-MRSA persistence due to transmission via pig movements alone. However, no comparisons were made to LA-MRSA screenings performed in Denmark. A second LA-MRSA model examined the possible spread on pig farms in the Swedish pig population, and evaluated the performance and cost of a proposed surveillance programme[83].

However, the herd prevalence in Sweden is at a very low level compared to that of Denmark, and results can therefore not be applied to the current Danish situation.

2. MATERIALS AND METHODS

2.1. NETWORK ANALYSIS (MANUSCRIPT I)

Information on pig herds and pig movements registered in the Central Husbandry Register (CHR) and the Pig Movement Database (PMD) in Denmark between 1st January 2006 and 31st December 2015 were used to build a network with pig herds as nodes and pig movements as links between the nodes. In total, 16,069 holdings and 7,617,681 pig movements were included in the analysis.

A general description of the underlying datasets was given in order to evaluate trends in the number of active herds and registered pig movements. The contact patterns between the registered holding types were described by counting the registered movements between all holding types. Static network analysis tools were used to determine the herd types in the network components such as the giant strongly connected component (GSCC), in which trade connections exist between all pairs of holdings. We also evaluated the loyalty patterns of registered holding types by measuring the fraction of preserved incoming and out-going trade connections over two consecutive years. Dynamic network analysis tools were used to calculate the size of incoming and out-going contact chains of different holding types by assessing how many herds were connected by trade within a certain time period. A detailed description of the materials and methods used can be found in Manuscript I (Chapter 3.1).

2.2. SIMULATION MODELLING (MANUSCRIPT II)

Information from the dataset used in the framework of the network analysis on all herds and corresponding pig movements between these herds of five holding types (breeding and multiplier herds, production herds, weaner herds, organic and free-ranging pig herds, and hobby herds) were used to mimic the spread of LA-MRSA among pig herds. In total, 12,874 herds and 993,474 pig movements were included in the study period from 1st January 2006 to 31st December 2015.

An agent-based Monte Carlo simulation model was developed. Within-herd dynamics, transmission via pig movements and indirect transmission among herds were implemented as stochastic processes. A three-compartment model was used to mimic the spread of LA-MRSA within and between the sow,

weaner and finisher sections of each herd. Higher transmission rates were assumed for herds using high-risk antibiotics compared to herds that did not receive prescriptions for β-lactams or tetracycline. Pigs could be cleared of LA-MRSA with a pre-defined probability at any time during the simulation. Pig movements were modelled based on the registered movements in the PMD. The number of LA-MRSA-positive pigs within the batch of moved pigs was estimated based on the prevalence in the sending herd. Indirect contact between pig herds was modelled via (1) contact with humans, (2) trucks picking up pigs for the abattoir and (3) contact between herds with the same owner. Model parameters were taken from the literature and expert opinions. An extensive sensitivity analysis was performed to study the effects of variations in the model parameters on the model outcome.

Several initialisation scenarios were simulated to mimic different routes of LA-MRSA introduction in the pig population and the predicted median herd prevalence on 31st December 2008 and 2014 was compared to the results of LA-MRSA screenings performed in Denmark.

A detailed description of the model and the scenarios is presented in Manuscript II (Chapter 3.2.)

2.2.1. Additional methods (not presented in Manuscript II)

To further quantify the impact of the modelled pathways of LA-MRSA transmission among pig herds, we investigated how often transmission occurred per 30 days for each of the modelled transmission routes: (1) animal movements, (2) indirect contact between pig herds via humans (visitors, veterinarians and advisors), (3) abattoir movements and (4) common ownership. In the simulations, records were kept on which transmission route affected each herd, and the number of herds that were newly affected by LA-MRSA via each transmission route. We summed up these numbers for 30 days and calculated the proportion of each transmission pathway from the total number of transmissions.

We evaluated the transmission routes for the default scenario, where LA-MRSA was initialised in 100 production herds in 2006, in one breeding and multiplier and 100 production herds in 2009. In addition, we investigated six scenarios - run in the framework of the sensitivity analysis - related to indirect transmission routes, in which (1) the frequency of indirect contact from indoor and outdoor herds, (2) the probability of effective transmission via indirect contact, and (3) the probability of transmission via abattoir movements were both halved and doubled (Table 3 in Manuscript II, Chapter 3.2).

2.3. Modelling control options (Manuscript III)

We enhanced the model presented in Manuscript II (Chapter 3.2) with four potential control options:

(1) A reduced proportion of herds using high-risk antibiotics: two scenarios mimicking 50% or 100% of active herds terminating their use of high-risk antibiotics. We simulated LA-MRSA spread within these herds with lower transmission rates.

(2) Increased biosecurity: the probability of LA-MRSA transmission among pig herds via human contact was reduced by 50% or 75% to mimic increased biosecurity.

(3) Trade restrictions prohibiting trade from LA-MRSA-positive to negative herds: the LA-MRSA status was determined one or four times per year for all herds. Based on these simulated test results, pig movements from LA-MRSA-positive herds to herds tested negative were ignored.

(4) Voluntary eradication: after simulating an LA-MRSA screening in all herds one or four times per year, 7.5% or 5% of herds were randomly chosen to start an eradication process. A stand-still scenario was modelled for these herds over a pre-defined time period. All incoming and out-going pig movements registered during the stand-still were ignored and the herd status after eradication was set to LA-MRSA negative.

All control options and their combinations were initialised on 1st January 2007 or 1st January 2010. We compared the predicted herd prevalence 6 years after control measures began to the scenario without control measures. The relative reduction was also compared between the two control start dates to evaluate the influence of the predicted herd prevalence at the start of control measures on the reduction rates.

A more detailed description is included in Manuscript III (Chapter 3.3).

2.3.1. Additional methods (not presented in Manuscript III)

The predicted median herd prevalence 6 years after initialisation of LA-MRSA in 2007 is presented in Manuscript III. The herd prevalence on 31st December 2015 was also predicted to evaluate the effects of three additional years of active control measures.

Selecting herds identified as hubs for disease spread for eradication might offer the potential for a further reduction of LA-MRSA spread among pig holdings. However, herds that started the voluntary eradication process were selected randomly in Manuscript III.

The size of the out-going contact chain was calculated for each herd every year as part of the framework of the analysis in Manuscript I (Chapter 3.1). These values were added to the input data and could be used in the simulation model. After screening herds for LA-MRSA in the simulation model, all breeding and multiplier herds (other herds) that tested positive for LA-MRSA were ordered according to the size of the out-going contact chain. The 7.5% (5%) of breeding herds (other herds) that tested positive in the simulation with the largest out-going contact chains were then defined as hubs and chosen to start the eradication process.

All control options were repeated using a scenario in which LA-MRSA was initialised in 10,000 randomly chosen active herds on 26th December 2006. Our goal was to mimic a situation where the herd prevalence was at a high level at the end of 2006, just before control measures were started. This would allow us to

study the effects of control measures from a high initial prevalence, thus mimicking the current situation in Denmark, where the between-herd prevalence is high. In this scenario, there was no second introduction in 2009, as in the earlier scenarios.

If movement restrictions prohibited the movement of pigs from LA-MRSA-positive to negative herds in the model, no alternative movement of those pigs was simulated. We assumed that these animals might be exported due to the movement restrictions. In addition to recording the reduction in median herd prevalence as a consequence of the implemented control measures for each scenario, we also recorded the number of ignored movements due to movement restrictions, the number of pigs sent to slaughter due to the eradication process, and the number of herds that started the eradication process.

3. MANUSCRIPTS

3.1. MANUSCRIPT I: NETWORK ANALYSIS OF PIG MOVEMENTS: LOYALTY PATTERNS AND CONTACT CHAINS OF DIFFERENT HOLDING TYPES IN DENMARK

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RESEARCH ARTICLE

Network analysis of pig movements: Loyalty patterns and contact chains of different holding types in Denmark

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Abstract

Understanding animal movements is an important factor for the development of meaningful surveillance and control programs, but also for the development of disease spread models. We analysed the Danish pig movement network using static and temporal network analysis tools to provide deeper insight in the connection between holdings dealing with pigs, such as breeding and multiplier herds, production herds, slaughterhouses or traders. Pig movements, which occurred between 1st January 2006 and 31st December 2015 in Denmark, were summarized to investigate temporal trends such as the number of active holdings, the number of registered movements and the number of pigs moved. To identify holdings and holding types with potentially higher risk for introduction or spread of diseases via pig movements, we determined loyalty patterns, annual network components and contact chains for the 24 registered holding types. The total number of active holdings as well as the number of pig movements decreased during the study period while the holding sizes increased. Around 60–90% of connections between two pig holdings were present in two consecutive years and around one third of the connections persisted within the considered time period. Weaner herds showed the highest level of in-loyalty, whereas we observed an intermediate level of in-loyalty for all breeding sites and for production herds. Boar stations, production herds and trade herds showed a high level of out-loyalty. Production herds constituted the highest proportion of holdings in the largest strongly connected component. All production sites showed low levels of in-going contact chains and we observed a high level of out-going contact chain for breeding and multiplier herds. Except for livestock auctions, all transit sites also showed low levels of out-going contact chains. Our results reflect the pyramidal structure of the underlying network. Based on the considered disease, the time frame for the calculation of network measurements needs to be adapted. Using these adapted values for loyalty and contact chains might help to identify holdings with high potential of spreading diseases and thus limit the outbreak size or support control or eradication of the considered pathogen.

study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Introduction

The movement of pigs between holdings is an important route of transmission for pathogens [1,2]. Therefore, trade restrictions are implemented in case of an outbreak of any highly contagious disease, e.g. foot and mouth disease and classical swine fever [3]. However, other pathogens such as livestock associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) do not invoke movement restrictions. Hence, these pathogens might spread freely via pig movements.

Facilities such as production herds, slaughterhouses and traders form a complex network. During the last decade, methods of network analyses were described and introduced into veterinary science [4,5,6,7]. Network analysis helps characterising the contacts between holdings and leads to a better understanding of the potential risk for the spread of pathogens through the production chain. Exploring livestock movements can contribute towards risk-based control strategies to prevent and monitor the introduction and the spread of infectious diseases in animal populations.

Since the EU regulations require identification and registration of pigs [8], data on pig transports are available on a large scale. Network analyses of pig movements were performed in several countries and highlighted the potential for pathogen spread and implications for control programs by estimating the potential transmission pathways between holdings connected by direct or indirect contacts [9–14].

The Danish pig industry is among the leading pig industries in the world in areas such as breeding, quality, food safety and traceability. As a result, the Danish pig industry constitutes an essential part of the Danish economy, as approximately 85% of Danish pork and 13 million pigs are exported every year [15]. The Danish pig production is pyramidal structured with breeding sites on the top, production sites in the centre, down to end of production sites like slaughterhouses in the bottom of the pyramid [16] (Fig 1).

In Denmark, Bigras-Poulin et al. [16] described trade patterns of Danish pigs between Sept 2002 and May 2003. Their analysis covered only a short time period and thus actual temporal trends for the development of the pig production sector could not be gained. Nevertheless, long term analysis is essential to understand the dynamics of disease spread in complex networks such as the pig movement network in Denmark [16]. With this information the consequences of the introduction of a contagious disease can be estimated and control measures can be planned. If holdings differ from one another with respect to their potential to spread diseases, this variability can be used to rank the holdings. Such a ranking allows veterinary authorities to select holdings or holding types for the implementation of targeted surveillance and control measures.

In this study, our main goal was to provide a comprehensive description and exploration of changes over time in the structure of the Danish pig movement network in the period from 1st January 2006 to 31st December 2015 by use of recently developed approaches of static and temporal network analysis tools. Descriptive statistics of parameters such as the number of active holdings, the number of registered pig movements and the number of pigs moved between different types of pig holdings are presented. To identify holdings and holding types with potentially higher risk for introduction or spread of diseases via pig movements, we calculated the holdings in- and out-loyalty as a local measure of its tendency to maintain contacts with the same holdings over time. Furthermore, we extracted network components to identify subsets of holdings where connectedness is particularly high and the holding types most prevalent within connected components. Finally, we evaluated the size of the in-going and out-going contact chain for each holding by tracing back and forward all direct and indirect pig movement contacts within yearly snap shots. These investigations could be used to develop

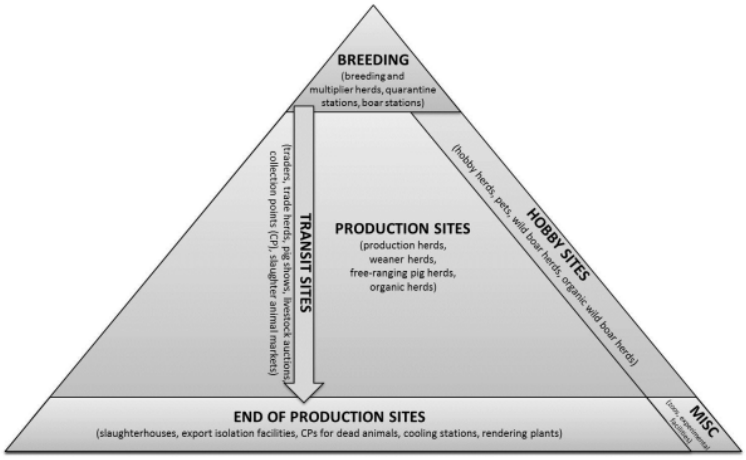

Fig 1. Pyramidal structure of the Danish pig production chain. The 24 holding types considered in this study were assigned to generic production steps. Besides the vertical connections, illustrated by the vertical transit sites horizontal connections also exist.

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meaningful surveillance and control programs, by focussing on high risk holdings or a holding type for surveillance or monitoring. Additionally, results gained by network analysis could be used for the development of disease spread models for the simulation of movement patterns in case of missing real data.

Material and methods

Data set

In Denmark, information on pig movements is part of the Central Husbandry Register (CHR) [17,18]. This central database was established in 1992 and is owned by the Ministry of Environment and Food. Data from 1st January 2006 to 31st December 2015 representing all registered pig holdings and pig transports between holdings in Denmark were used in this study. The basis for our analysis consisted of 18,648 holdings registered in the CHR within the considered time period. The CHR data provided among others, holding and enterprise identification number, information on holding type and the number of sows, finishers and weaners in each holding. The CHR data were available on yearly basis, and thus changes in holding types and the number of pigs in each holding were accessible. There are 24 pig holding types predefined in the CHR, and the owner has to indicate the type of holding during the registration process (Fig 1). No definitions of the types of holdings are available in the CHR. In Denmark, several holdings of different types can be owned by one person and thus constitute an agricultural enterprise (farm). We performed all analyses at the holding level.

The movement data file contained 7,678,851 movement records. For each pig movement data record, sending and receiving holding and enterprise identification numbers, the number of moved pigs and the date of the pig movement are recorded. Movements among holdings owned by the same farmer are recorded and thus included in the study. As holdings within each farm can be of different types, e.g. production herd and cooling station, both holding and

enterprise identification numbers were used in the analysis. From this point, we define "holding" as identified by the combination of holding and enterprise number in the CHR.

Imports to and exports out of Denmark were not included in the movement dataset. In total, 8,949 movements were excluded, because the sending (7,972) or receiving (977) holding was not registered as a pig holding but kept other species. One holding and 51,820 corresponding movements were excluded from the analysis due to mistakes during the registration of movements. Additionally, 401 out-going movements from slaughterhouses were excluded, because the receiving holding type was not a slaughterhouse or rendering plant. On follow-up investigations of these excluded out-going movements, many turned out to be registration errors. As movements out of slaughterhouses to production herds are not allowed to occur, these movements were excluded. Movements between slaughterhouses occur in case of a lack of capacity in the sending slaughterhouse. Also movements of dead animals to e.g. rendering plants are recorded in the movement database. For the sake of a complete description of the Danish pig movement network, we kept these records. We only included holdings that were at least once registered as sender or receiver of pigs, leading to 16,069 holdings and 7,617,681 movements of pigs included in the analysis.

Data analysis

For 5,147 holdings, the type of holding was not registered in the data set. These holdings were checked manually at the CHR website and if active on 4 Jul 2016 the holding type at that date was used in the analysis. For 2006, the number of weaners was not available in the CHR. We therefore estimated the number of weaners by multiplying the given number of sows with 4.5 based on production results in the swine industry [19]. The size of the holdings was calculated as the sum of the registered number of sows, finishers and weaners. If no sows, finishers and weaners were registered, the total holding size was set to "not available".

Data summaries. Holdings were assumed to be active within a year, if the holding was involved in at least one pig movement. We determined the annual number of active holdings and the annual number of registered pig movements in order to describe the changes over time. Additionally, we calculated (1) the number of holdings per type over the years, (2) the median number of pigs moved each year by type of holding for both the sending and receiving holding, and (3) the median number of pig movements per holding type, again for both the sending and receiving holding.

To show the connection between holdings of different holding types, we generated heat maps showing (1) the number of movements occurring between different holding types and (2) the number of pigs moved between two different holding types for the whole time period and yearly snapshots.

Static network analysis. A network was generated using the pig holdings as set of nodes V that are connected by pig movements as set of links E. Thus a link exists between two premises, if at least one pig movement was recorded in the whole time period from 2006 to 2015. The network is directed, meaning that a holding could be linked one-sided (if the holding only sends or receives pigs from another holding) or in both directions (if the holding sends and receives pigs from another holding). Additionally, yearly snapshots containing only active pig holdings and movements occurring in the considered year were generated to analyse the network over time.

To describe the level of variation of the network on a yearly basis, we calculated the network loyalty defined as the fraction of common directed links for all considered years [20]. Therefore, we calculated a yearly adjacency matrix A^t. For a network with a set of nodes V, the adjacency matrix A^t is a square $|V| \, x \, |V|$ matrix with A^t_{ij} is one when there is an directed link from

holding i to holding j in year t, and zero when there is no link in year t. The network loyalty Λ_{t_1,t_2} is then defined as the count on how often $A_{ij}^{t_1} = A_{ij}^{t_2} = 1$ divided by $|V^{t_1}|$. The memory in link occurrence can be quantified using network loyalty for all possible time differences. More specifically, we define the link memory of a network as the average number of common links between all year-pairs (t_1,t_2), where $t_1 < t_2$ and where the memory length is measured in terms of $\tau = t_2 - t_1$.

Node loyalty θ measures the fraction of preserved direct contacts (neighbours) of a holding between two consecutive years, $t-1$ and t. [20]. In order to quantify $\theta_i^{t-1,t}$, we define $\mathbf{Y}_i^{t-1}$ as the set of in- and out-going neighbours of holding i in year $t-1$. Then $\theta_i^{t-1,t}$ is given by the Jaccard Index

$$\theta_i^{t-1,t} = \frac{|\mathbf{Y}_i^{t-1} \cap \mathbf{Y}_i^t|}{|\mathbf{Y}_i^{t-1} \cup \mathbf{Y}_i^t|}$$

We expanded this concept to in- and out-loyalty which measures the fractions of preserved direct neighbours (in-loyalty ${}^{in}\theta$) from which a holding receives pigs (${}^{in}\mathbf{Y}i^{t-1}$) or the fraction of preserved direct neighbours (out-loyalty ${}^{out}\theta$) to which a holding sends pigs (${}^{out}\mathbf{Y}i^{t-1}$). Both, in- and out-loyalty were determined for all holdings in the network, the yearly snapshots and for each type of holding to compare holding types. Therefore, we aggregated the yearly in- and out-loyalty values for each holding type

$${}^{in}_{ht}\theta = \bigcup_{\substack{t=2007 \\ i \epsilon V_k^t}}^{2015} {}^{in}\theta_i^{t-1,t} \quad and \quad {}^{out}_{ht}\theta = \bigcup_{\substack{t=2007 \\ i \epsilon V_k^t}}^{2015} {}^{out}\theta_i^{t-1,t},$$

provided that V_k^t is defined as a subset of all holdings at time $t \epsilon$ 2007, . . . 2015 of the considered type of holding k. To categorize the holding types, three levels for in- and out-loyalty ${}^{in}_{ht}\theta$ and ${}^{out}_{ht}\theta$ were used. If the average loyalty of all holdings of holding type ht was below 0.45 (between 0.45 and 0.55, or above 0.55), the holding type was categorized as holding type with low (intermediate, or high) loyalty.

To characterize the network, we computed the fragmentation F, which measures the number of paths in different components over the number of all possible paths in the network. A connected component is defined as subset of nodes $C \subseteq V$ for which a path exists between any pair of nodes in C. A path between two nodes is a direct or indirect connection between them. The fragmentation is calculated as

$$F = 1 - \frac{\sum_k S_k(S_k - 1)}{n(n-1)},$$

with S_k the number of holdings in component k and n the total number of holdings in the network. The fragmentation ranges between 0 and 1. A value close to 0 indicates a very connected network and a value of 1 means that every node is isolated [21].

A network component analysis was performed for the whole static network and for the yearly snapshots to identify subsets of nodes in the networks where connectedness is particularly high [21]. The general component structure of directed networks was investigated by Dorogovtsev et al. [22]. The giant strongly connected component (GSCC) is the largest subset of holdings where there exists a path between all pairs of holdings in that subset. It is a particular feature of directed networks that the existence of a giant component induces the existence of other groups of holdings. These are (1) the giant in-component (GIC), which consists of all

holdings with out-going contacts to the GSCC that they are not part of, and (2) the giant out-component (GOC), which consists of all holdings receiving pigs directly or indirectly from the GSCC that they are not part of [12,22]. Additionally, we determined the holding types within the three types of components.

Temporal network analysis. In contrast to the static situation, the time when contacts between holdings occur and especially the chronological order of contacts is taken into account in temporal networks.

We calculated the size of the in-going and out-going contact chain for each holding, by tracing back and forward all direct and indirect contacts within the yearly snap shots [20, 21]. Both measurements could be useful when setting up strategies for disease control as they identify holdings with many contacts through pig movements and that thus are at potentially higher risk for introduction or spread of diseases.

Software

Data processing and network analysis was done in R version 3.2.2 (R Development Core Team, 2015)—"Fire Safety" using the packages igraph [23] and epiContactTrace [24].

Results

Data analysis

Data summaries. In total, there were 24 different types of holdings in the dataset. Of the 12,814 holdings in 2006 to 7,835 holdings in 2015, 91.6to 79.5% were registered as production herds (Table 1). The total number of active holdings decreased over the considered time period. The number of active production herds also decreased, whereas the number of other holding types such as breeding and multiplier herds and boar stations remained constant. The number of weaner herds and hobby herds increased over time (Table 1).

The median holding size increased between 2006 and 2015 (Fig 2, S1 File). The number of holdings in the category "holding size not available" was not equally distributed over time and ranged between 3% in 2008 and 15% in 2014. Breeding and multiplier herds, production herds and weaner herds were the holding types with the highest median holding sizes (S1 File).

The overall frequency of pig movements decreased over time (Fig 2) at all levels of aggregation. Fewer movements are recorded on weekends and bank holidays. The weekly aggregated movements (7 days) showed occasional weeks with fewer movement frequencies reoccurring each year due to holidays. When looking at yearly pig movements by type of holding, the results showed: (1) a constant average number of movements were recorded out of breeding and multiplier herds and production herds, (2) a reduction of the average number of movements to slaughterhouses and rendering plants, (3) a reduction of the movements to transit sites, and (4) an increase in movements from weaner herds (S2 File).

The median number of pigs moved in one movement (batch) increased in the period between 2006 and 2015 (Fig 2, S2 File). The annual values for the maximum batch sizes were very high (mean = 8,057, ranging from 6,918 to 9,780).

Transports from production herds to slaughterhouses and rendering plants represented 77% of the registered pig movements (Fig 3). Looking at the number of pigs moved between two holding types, 78% of the pigs were moved from production herds to (1) production herds, and (2) slaughterhouses (Fig 3). Additionally, 15% of the pigs were moved from breeding and multiplier herds to production herds, between production herds and weaner herds and from production herds to collections points. These movements reflect the pyramidal structure of the pig production sector.

Table 1. Number of active Danish pig holdings.

	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Breeding sites										
Breeding and multiplier herds	278	261	252	263	270	276	272	271	260	234
Quarantine stations	71	75	62	57	48	39	46	41	36	44
Boar stations	18	17	16	15	16	16	17	17	17	17
Production sites										
Production herds	11,733	10,867	9,902	8,691	7,940	7,538	7,090	6,819	6,623	6,230
Weaner herds	110	105	106	171	241	240	227	224	223	201
Free-ranging pig herds	325	279	189	190	157	145	141	153	169	164
Organic pig herds	76	79	117	106	99	93	90	87	90	88
Hobby sites										
Hobby herds	69	101	110	196	358	391	454	561	721	521
Pets	5	2	1	1	2	2	1	3	10	11
Wild boar herds		2	1	1	1	1	15	8	8	11
Organic wild boar herds										1
Transit sites										
Traders	7	6	10	8	8	5	6	7	8	3
Trade herds		1	1	7	20	17	17	16	15	16
Pig shows	9	8	8	6	6	6	9	9	6	7
Livestock auctions	1	1								
Collection points (CP)	7	11	15	16	17	17	20	21	24	25
Slaughter animal markets	1	2	2					1	1	1
Miscellaneous										
Zoos						1	1	4	4	4
Experimental facilities			1	1	3	3	2	1	2	5
End of production sites										
Slaughterhouses	100	97	89	83	80	78	74	75	77	76
Export isolation facilities						3	3	3	3	3
CPs for dead animals	2	4	2	2	2	2	7	18	35	121
Cooling stations	1	1	1	1	1	1	9	30	33	51
Rendering plants	1	1	1	1	1	1	1	1	1	1
Total	12,814	11,920	10,886	9,816	9,270	8,875	8,502	8,370	8,366	7,835

Number of active Danish pig holdings (sent or received pigs at least once) from 1st January 2006 to 31st December 2015 in Denmark.

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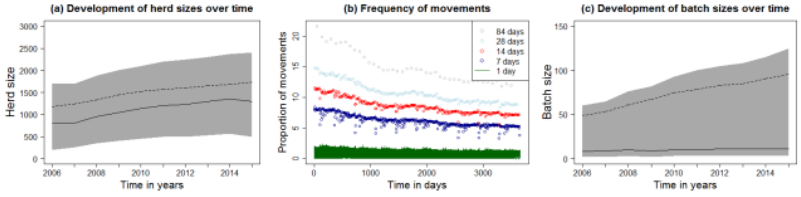

Fig 2. Descriptive statistics of the examined data. Figure (a) and (c) show the median (solid black line) and average (dashed black line) of holding sizes and of the number of pigs moved per pig movement sizes over time. Grey areas represent the range between 1st and 3rd quantile. Temporal trends of holding sizes for the investigated 24 holding types are shown in S1 File. Figure (b) shows the proportion of registered pig movements out of all possible movements in Denmark between 1st January 2006 and 31st December 2015 on daily base (green) and aggregated for 7 (dark blue), 14 (red), 28 (light blue) and 84 (grey) days.

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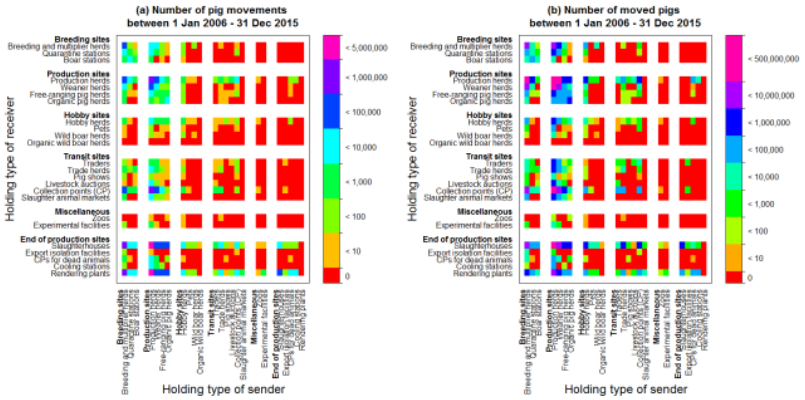

Fig 3. Registered pig movements and number of pigs moved. Heat maps describing (a) the number of registered pig movements and (b) the number of pigs moved between holding types from 1st January 2006 and 31st December 2015 in Denmark. The heat maps show stability over time and for the number of movements (data not shown).

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Static network analysis. Around 60–90% of connections between two pig holdings were present in two consecutive years and around one third of the connections persisted the full period from 1st January 2006 to 31st December 2015 (Fig 4). Fig 4b shows the memory of common links between pairs of consecutive years. For short time differences, the number of common directed links decreases faster compared to longer time differences.

Comparing the values for node loyalty for each pair of consecutive years over time, we observed a slight shift to higher in- and out-loyalty over time (Fig 5, S3 File). In general, there were more holdings with in-loyalty equal to 1 compared to out-loyalty equal to 1. Table 2 summarizes the in- and out-loyalty per holding types (see also S3 File). Weaner herds showed the highest level of in-loyalty, whereas we observed an intermediate level of in-loyalty for all breeding sites and production herds (S3 File). Boar stations and production herds showed a high level of out-loyalty.

The 10 years network showed a fragmentation index of 0.86. Thus the network is only connected to a low degree. Nevertheless, it contained (per definition) no isolated holdings. The

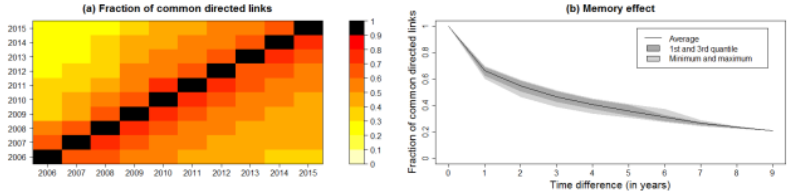

Fig 4. Fraction of common directed links. (a) Network loyalty (fraction of common directed links contained in two consecutive snapshots) of the pig movement network in Denmark between 1st January 2006 and 31st December 2015. Asymmetry is caused by varying number of directed links per year. (b) Development of the fraction of common directed links per year time difference. Dark grey areas represent the interval between 1st and 3rd quantile, light grey areas represent the interval between minimum and maximum values for the fraction of common directed links.

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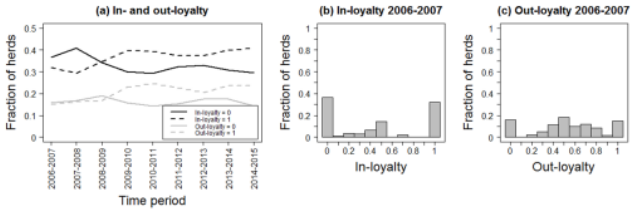

Fig 5. In- and out-loyalty patterns. (a) Fraction of holdings with in-loyalty and out-loyalty equal to 0 and 1 for the whole network of pig movements from 1st January 2006 to 31st December 2015 in Denmark. (b) and (c) show the histograms of in- and out-loyalty for the two consecutive years 2006 and 2007. Both histograms refer to the years 2006 and 2007 for visualization purposes, all other distributions show stability over time and are shown in in S2 File.

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fragmentation indices for the yearly network snapshots were 0.99 in all considered time periods. The sizes of the different GSCCs included less than 1% of active holdings each year (Fig 6), with the largest GSCC recorded in 2008 with 55 holdings. We observed variations between years in the size of the GIC, ranging from 1,893 holdings in 2006 to 2 holdings in 2007 (Fig 6). The size of the GOC was below 20% except in year 2013 (Fig 6).

Production herds constituted the highest proportion of holdings in the GSCC and were part of the GSCC in every year except of 2011 (Table 3). Breeding and multiplier herds were the only holding type that was part of the GIC in each year. Production herds, slaughterhouses and rendering plants were part of the GOC in every year, but also breeding and multiplier herds were part of GOC in 2011 and 2013.

Temporal network analysis. We calculated the size of the in-going and out-going contact chains for the whole network from 2006 to 2015 (S4 File). In total, the values of the in-going contact chains were higher compared to the size of the out-going contact chains over the study period of 10 years. The size of the in-going and out-going contact chains varied between holding types (Fig 7, Table 2, and S4 File). All production sites showed low levels of in-going contact chains, whereas the levels of out-going contact chains vary. Quarantine and boar stations showed intermediate levels of in-going contact chains and we observed a high level of out-going contact chain for breeding and multiplier herds. Except of livestock auctions, all transit sites also show low levels of out-going contact chains.

Discussion

Data set

Although there have been many studies analysing pig movement networks, most covered only time periods of one to three years [12,25,26,27]. The presented study describes the development of the number of active pig holdings, holding sizes and the network of pig movements in Denmark over a period of 10 years from 1st January 2006 to 31st December 2015, and hence presents long term changes.

The pig movements between holdings included in this study comprised over 99% of the movement records in the database. Only holdings not registered as pig holdings in the CHR and registration errors in the movement records were excluded. The registration errors mainly were related to out-going movements of slaughterhouses. As slaughterhouses are dead ends of the production chain [28] they do not play a key role in disease spreading. Exclusion of these records is therefore not expected to influence the overall results of this study.

Table 2. Levels of loyalty and contact chains for different pig holding types in Denmark, based on data from the CHR, 1st January 2006 – 31st December 2015.

	In-loyalty	In-going contact chain	Out-loyalty	Out-going contact chain
Breeding sites				
Breeding and multiplier herds	intermediate	low	intermediate	high
Quarantine stations	intermediate	intermediate	intermediate	intermediate
Boar stations	intermediate	intermediate	high	low
Production sites				
Production herds	intermediate	low	high	intermediate
Weaner herds	high	low	intermediate	intermediate
Free-ranging pig herds	low	low	low	low
Organic pig herds	low	low	intermediate	low
Hobby sites				
Hobby herds	low	low	low	low
Pets	low	intermediate	high	low
Wild boar herds	low	low	low	low
Organic wild boar herds	not available	low	not available	low
Transit sites				
Traders	low	intermediate	low	low
Trade herds	low	intermediate	high	low
Pig shows	low	low	low	low
Livestock auctions	low	intermediate	low	high
Collection points (CP)	low	intermediate	intermediate	low
Slaughter animal markets	low	intermediate	low	low
Miscellaneous				
Zoos	low	low	low	low
Experimental facilities	low	low	low	low
End of production sites				
Slaughterhouses	low	high	high	low
Export isolation facilities	low	intermediate	high	low
CPs for dead animals	intermediate	low	intermediate	low
Cooling stations	high	low	high	low
Rendering plants	high	high	not available	low

Levels for in- and out-loyalty: low: mean < 0.45, intermediate: 0.45 $\leq$ mean $\leq$ 0.55, and high: mean > 0.55. Levels of the size of in-going and out-going contact chains: low: $m_{ht} < 10$, (2) intermediate: $10 \leq m_{ht} \leq 50$, and high: $m_{ht} > 50$ with m_{ht} as mean value of the annual sizes of in-going and out-going contact chain per holding type.

https://doi.org/10.1371/journal.pone.0179915.t002

The holding types are self-reported by the owner of the holding. As there are no definitions of holding types available, misclassification is likely. Additionally, available holding types in the CHR changed over time. The holding type "livestock auctions" does not exist anymore since 2008, as pigs are no longer sold at auctions. The holding type "organic wild boar" was introduced in 2015. Breeding and multiplier herds are summarized in one category. Breeding herds are not expected to have in-going contacts and differ from multiplier herds regarding herd management. This fact becomes important when developing control programs for different holding types. Furthermore, the validity of the CHR has increased over the time period, as the CHR data has been merged with other types of data with the purpose of decreasing the use of antimicrobials [29], which might influence the number of herds in each holding type.

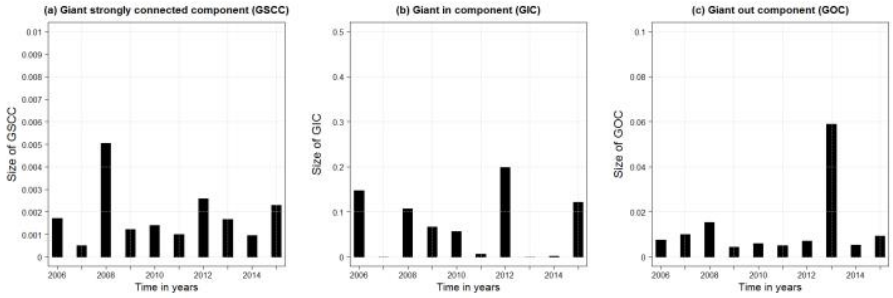

Fig 6. Pig movement network component sizes (proportion of overall number of active holdings). Sizes of (a) the giant connected components (GSCC), (b) the giant in components (GIC) and (c) the giant out components (GOC) for the yearly snapshots of the pig movement network in Denmark between 2006 and 2015.

https://doi.org/10.1371/journal.pone.0179915.g006

It might be useful to add clear definitions for the holding types to the CHR and restrict the use of some holding types dependent on other available information such as the registration of the holding as specific pathogen free herd (SPF). Such definitions might also include information on the biosecurity level of the holding. Information on biosecurity in Danish pig holdings are currently only available for SPF herds, and registered in the private SPF-register. Furthermore, a non-SPF status cannot be interpreted as low level of biosecurity, as many holdings have high biosecurity standards, even if not enrolled in the SPF system. However, implementation of biosecurity in the CHR register could add important information regarding risk of disease introduction and spread. However, the effect of biosecurity depends on a daily continual awareness of biosecurity procedures. Self-reporting of the level of biosecurity might lead to overestimation of one's own performance, like the lack of control might lead to a slowly decrease in the producer's awareness and performance regarding biosecurity. Official veterinarians could perform regular inspections and use already available methods to assign a biosecurity level to each holding.

Data analysis

Data summaries. We estimated the number of weaners per holding in 2006, because these values were not given in the data. The estimated holding sizes fit into the observed trend, even though higher values have been reported from other countries for estimates of the number of weaners based on the number of sows per holding [30]. Nevertheless, these values might have changed since 2006.

We observed a decreasing number of holdings, but an increasing size of the holdings in the considered time period. Nöremark et al. [9] observed the same trend in Sweden. The decreasing frequency of movements is most likely due to the decreasing number of active holdings during the study period. Lentz et al. [12] observed similar patterns for the frequency of movements for a German pig movement network. In addition, the median size of batches of pigs moved remained constant.

The maximum batch sizes were very high in each year and this could be attributed to errors in reporting: movements could have occurred over a certain time period but were all reported on a single day. Nevertheless, larger batches might increase the probability of transferring a disease from one holding to another.

The constant average number of out-movements from breeding and multiplier herds and production herds in combination with the reduction of movements to slaughterhouses and

Table 3. Number of years between 2006 and 2015, in which the holding type is present in the pig movement network components.

	Giant strongly connected component (GSCC)	Giant in component (GIC)	Giant out component (GOC)	Average number of active herds
Breeding sites				
Breeding and multiplier herds	3 (3)	10 (91)	2 (5)	264
Quarantine stations	1 (4)	7 (10)	4 (6)	52
Boar stations	1 (4)	4 (4)	3 (11)	17
Production sites				
Production herds	9 (15)	8 (708)	10 (74)	8343
Weaner herds	2 (1)	7 (34)	2 (6)	185
Free-ranging pig herds	4 (1)	6 (7)	4 (4)	191
Organic pig herds		3 (4)	4 (2)	93
Hobby sites				
Hobby herds	1 (1)	4 (3)	7 (5)	348
Pets			2 (1)	4
Wild boar herds		1 (1)		5
Organic wild boar herds				1
Transit sites				
Traders	2 (2)	4 (2)	5 (1)	7
Trade herds	2 (1)	2 (3)	2 (4)	12
Pig shows			2 (2)	7
Livestock auctions	1 (1)		1 (1)	1
Collection points (CP)	6 (2)	3 (1)	9 (5)	17
Slaughter animal markets			3 (2)	1
Miscellaneous				
Zoos			1	3
Experimental facilities				2
End of production sites				
Slaughterhouses			10 (24)	83
Export isolation facilities			1 (1)	3
CPs for dead animals				20
Cooling stations			1 (1)	13
Rendering plants			10 (1)	1
Average component size	18	529	120	

Number of years of the considered time period of 10 years from 2006 to 2015 in which a certain holding type was part of the giant strongly connected component (GSCC), the giant in component (GIC) and the giant out component (GOC). Values in brackets show the average number of holdings present in the component. Additionally, the average number of active herds is shown.

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rendering plants could be explained by the increased export. Furthermore, the reduction of movements to transit sites might influence the risk of introduction and spread of diseases. The increase in movements from weaner herds might describe a trend towards a more specialized production with more locations.

The type of holding highly influenced the frequency of contacts with other holdings as well as to which type of holding contacts occurred, which reflects the pyramidal structure of the Danish pig production sector. Lindström et al. [26] highlighted with a simulation study that these contact patterns might result in substantial differences in disease transmission via animal movements, depending on the index holding.

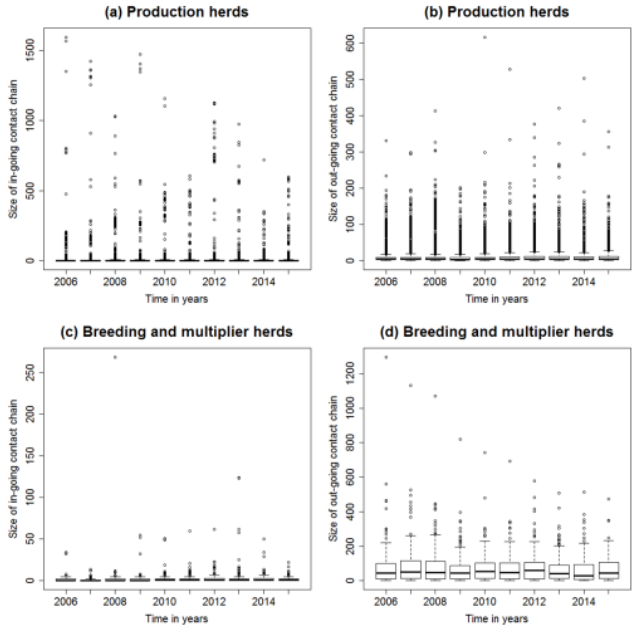

Fig 7. Contact chains for production and breeding and multiplier herds. Boxplots of (a) in-going and (b) out-going contact chains for production herds and (c) in-going and (d) out-going contact chains for breeding and multiplier herds from 2006 to 2015.

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Network analysis. Both, static and temporal network analysis approaches were used to describe the Danish pig movement data, as the static view alone does not take the dynamic aspects of contact patterns into account [31].

There are several methods available in both, static and temporal network analysis. We investigated in- and out-loyalty and in-going and out-going contact chains for 24 holding types. Other static network approaches such as in- and out-degree were shown to be less informative to describe the potential risk of a holding in spreading or contracting a disease [32]. Indirect in- and out-going contacts should be taken into account to better evaluate the risk of each holding becoming infected or spreading infections [33]. Thus, in this study we calculated the in-going and out-going contact chain taking the temporal order of pig movements into account. Additionally, [31,33] describe the out-going infection chain as more reasonable estimate of a potential maximal epidemic size. Nevertheless, we used a time frame of one year to calculate the contact chains. If a certain disease is considered, the time frame needs to be adapted to a meaningful range reflecting the duration of the incubation and infectious periods of that disease.

The methodological framework for the analysis of temporal networks is still in a starting phase [34]. The concept of components is well understood in static networks. Even though it is still a challenge to transfer the idea to temporal networks, an understanding of the static component structure is useful to develop meaningful surveillance and control programs. The small sizes of the yearly GSCCs might result from the pyramid structure or so called tree-like structure of the Danish pig production sector and lead to a restriction of the size of disease

outbreaks [12]. Nevertheless, these estimates might be overestimated because time is not considered. Most animal movement networks are highly fragmented, even if longer time periods are considered [33].

Even though we observed variability of loyalty values between holdings of the same type, clear loyalty patterns for different holding types were found. All breeding sites showed an intermediate level of in-loyalty whereas there was more variation on the level of in-loyalty for production sites. This reflects different management structures of holding types, which should be taken into account when developing control programs to limit the spread of diseases. Hobby and transit sites show lower levels of in-loyalty and thus might be at higher risk for introduction of diseases. On the other hand, only trade herds show a high out-loyalty as the majority of out-going movements go either to slaughterhouses or rendering plants. All other transit sites showed lower levels of out-loyalty which could increase the potential of spreading pathogens via trade.

Also the sizes of in- and out-going contact chain vary between holding types. End of production sites (Fig 1) show the highest levels of in-going contact chains, whereas we observed low levels for production sites. Breeding sites had higher out-going contact chains compared to all other holding type categories. Both reflect the pyramidal structure of the underlying network. Büttner et al. [32] investigated the size of in-going and out-going contact chain for four pig producing holding types in northern Germany (multipliers, farrowing farms, finisher farms and farrow-to-finisher farms). The values are lower compared to the values observed in this present study. This might be due to the lower number of active holdings within the study area and the considered time period of 3 years in [32].

Implications for disease control

Bigras-Poulin et al. [16] described the Danish pig movement network as one where a pathogen can maintain itself and spread even at low prevalences and thus eradication of pathogens would be difficult. Our results support these findings as we found holdings with high values of out-going contact chains that might have a high potential of spreading pathogens in the pig movement network. Additionally, the yearly components indicate circular connections between holdings that might support pathogen perpetuation. Nevertheless, the true transmission probability of infectious diseases via pig movements depends also on disease and holding specific parameters (e.g. virulence, biosecurity level and farmers' behaviour). Moreover, one should take into account the holding types present in the GIC and GSCC, as (1) these holdings might reach more other holdings, (2) the potential of an introduction of a pathogen to the holdings in a GSCC is related to the size of the GIC and (3) the maintenance of a pathogen is related to the size of the GSCC.

Holdings with a combination of a low/intermediate out-loyalty and high level of out-going contact chains might have a higher risk of spreading diseases compared to holdings with a high out-loyalty and a low size of out-going contact chains and should thus be targeted by surveillance. In our study breeding and multiplier herds at the top of the production pyramid showed this combination of high risk potential for disease spreading. Danish breeding and multiplier herds have very high levels of biosecurity, and therefore a somewhat lower risk of introduction of pathogens. However, once the pathogen is introduced, the following risk of spread is high. In the SPF system, these herds have monthly veterinary visits with focus on herd health and biosecurity [35]. Holdings registered as quarantine stations and weaner herds showed an intermediate risk, which may have the potential for disease spread and maintenance. Naturally, quarantine stations are under special surveillance as to assure the animals are free from disease before entrance to boar stations or other holdings [36,37].

In-loyalty and the size of the in-going contact chain are linked to the risk of the introduction of diseases via pig movements. Thus, high/intermediate in-loyalty combined with a low level of in-going contact chains might present a smaller risk compared to low in-loyalty and a high level of in-going contact chains. Breeding and multiplier herds, production and weaner herds showed the described lower risk combination. Production herds showed a low risk of receiving and spreading of pathogens, nevertheless, they constituted the largest group in the GSCC and GOC. As holdings in these components are linked to a wide range of other holdings and holding types, the risk for spreading and receiving diseases via pig movements might be still remarkable.

As the Danish pig production system is organised in a pyramidal structure with breeding herds at the top and slaughter pig production at the bottom [16], the start of eradication and control could be started in breeding herds. Restricted trade between holdings tested positive to holdings tested negative for a considered disease, as in the SPF-system, might additionally reduce the risk of introduction and re-introduction. This approach was recommended for LA-MRSA in [38] and could also be applicable for other endemic diseases. Nevertheless, for each considered disease, the analysis needs to be adapted regarding the time period for which loyalty and contact chains should be calculated. Based on this temporal adaptation, new definitions for the categorization of holding types should be used.

Around one third of the connections between holdings persisted between 2006 and 2015, suggesting long lasting trade connections are in place. This may lead to compliance issues if the disease status of holdings needs to be considered before movements without legislation in place to restrict these movements. This could be especially true for endemic diseases: intensive information and financial compensation might help to increase the motivation to change to a supplier that tested negative to prevent disease spread from positive to negative herds. Nevertheless, investigating loyalty patterns of holdings might help to find trade connections of positive tested herds and thus limiting the spread of pathogens in the network.

As animal trade is not the only pathway for transmission of pathogens [1,39], focussing only on holdings showing a high potential of spreading a pathogen might not be successful. Thus if eradication of a certain disease is the purpose, potential infection via mechanisms other than animal movements should be considered. Simulation modelling of the spread of pathogens could be based on animal movements and take into account other transmission mechanisms. The aim of a simulation model could be to understand the spread of a pathogen, but also to develop and test meaningful surveillance and control programs based on simulation studies. A first and main step in order to develop a simulation model mimicking the spread of pathogens is to describe animal movements and understand the movement patterns.

Supporting information

S1 File. Holding sizes of active Danish pig holdings. The file includes supporting figures and tables related to the holding sizes:

1. Descriptive statistics of the trends (1) of the holding sizes of active Danish pig holdings (holdings that at least once send or receive pigs to or from another holding) between 1st January 2006 and 31st December 2015 in Denmark (Table 1),
2. Proportion of holdings active between 1st January 2006 and 31st December 2015 in Denmark, categorized by the size of holding (Table 2),
3. Holding sizes by holding types for (a) breeding sites, (b) production sites, (c) hobby sites, (d) transit sites, (e) miscellaneous sites, and (f) end of production sites (Fig 1),

4. Descriptive statistics of the trend of all 24 holding types (Tables 3–8).
(PDF)

S2 File. Batch sizes and number of pig movements per holding type. The file includes supporting tables related to the number of pigs moved per registered pig movement (batch size) and the number of pig movements per holding type:

1. Descriptive statistics of the development of the number of pigs per movement (batch size) of registered pig movements between 1st January 2006 and 31st December 2015 in Denmark (Table 1).
2. Median batch size by holding type of sending and receiving holding of registered pig movements between 1st January 2006 and 31st December 2015 in Denmark (Tables 2 and 3).
3. Average number of pig movements per holding type of sending and receiving holding per year between 1st January 2006 and 31st December 2015 in Denmark (Tables 4 and 5).
(PDF)

S3 File. In- and out-loyalty per holding type. The file includes supporting figures and tables related to the in- and out-loyalty of the investigated holding types:

1. In-loyalty for each pair of consecutive years for the whole network of pig movements from 1st January 2006 to 31st December 2015 in Denmark (Figure 1),
2. Out-loyalty for each pair of consecutive years for the whole network of pig movements from 1st January 2006 to 31st December 2015 in Denmark (Figure 2).
3. In- and out-loyalty for (a) breeding sites, (b) production sites, (c) hobby sites, (d) transit sites, (e) miscellaneous sites, and (f) end of production sites (Figures 3–8),
4. Descriptive summaries of in- and out-loyalty per holding type (Tables 1 and 2).
(PDF)

S4 File. Contact chains. The file includes supporting figures and tables related to the in-going and out-going contact chains:

1. Size of (a) in-going and (b) out-going contact chain for the whole pig movement network in Denmark from 1st January 2006 to 31st December 2015 (Figure 1).
2. In-going and out-going contact chains for (a) breeding sites, (b) production sites, (c) hobby sites, (d) transit sites, (e) miscellaneous sites, and (f) end of production sites (Figures 2–7).
(PDF)

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3.2. MANUSCRIPT II: DRIVERS FOR LIVESTOCK-ASSOCIATED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* SPREAD AMONG DANISH PIG HERDS – A SIMULATION STUDY

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DRIVERS FOR LIVESTOCK-ASSOCIATED METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS SPREAD AMONG DANISH PIG HERDS - A SIMULATION STUDY

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ABSTRACT

To gain insight into the rapid increase in the number of livestock-associated Methicillin-resistant *Staphylococcus aureus* (LA-MRSA)-positive herds in Denmark, we developed an individual-based Monte Carlo simulation model. We aimed to assess whether transmission of LA-MRSA via pig movements could explain the observed increase in the number of positive herds in Denmark, and to evaluate the effect of other between-herd transmission mechanisms. Pig movements alone were not sufficient to mimic the observed increase in LA-MRSA-positive herds in Denmark in any of the modelled scenarios. The model identified three factors that played important roles in the between-herd spread of LA-MRSA: (1) the within-herd dynamics, (2) the frequency and effectiveness of indirect transmissions, and 3) unexplainable introduction of LA-MRSA to swine herds. These factors can act as starting points for the development of LA-MRSA control programs in pig herds in order to limit the risk of its transmission to humans.

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a ubiquitous bacterium in humans and animals, and a common cause of minor skin infections that do not usually require treatment. However, both *S. aureus* and Methicillin-resistant *S. aureus* (MRSA), which is resistant to beta-lactam antimicrobials, can lead to severe infections that could result in death, especially in individuals with a suppressed immune status. Livestock-associated MRSA (LA-MRSA) was first described in the Netherlands in 2005, and a special clonal complex, CC398, was identified shortly after[1]. LA-MRSA has been found in humans, pigs and other animal species since 2005[2–9]. In 2006, the pathogen was found in isolates originating from Danish pig farms[10]. A survey conducted in 26 European countries by the European Food Safety Authority (EFSA) in 2008 found 3% of Danish production herds, but no Danish breeding herds, positive for LA-MRSA type CC398[2]. However, a national survey conducted in 2014 found a prevalence of 63% in breeding herds and 68% in slaughter pig herds[11]. LA-MRSA has received enormous media attention due to its zoonotic potential, both in Denmark and beyond the borders.

Danish pig production has a pyramidal structure[12,13]. LA-MRSA-positive breeding sites at the top of the pyramid pose a risk of spreading the pathogen via pig movements to production sites in the centre and down to sites like slaughterhouses at the bottom of the pyramid. Several studies have indicated that the transmission of LA-MRSA via animal movements is one of the main drivers for its spread among pig herds[14–16]. Other transmission routes described are: air, housing environment, rodents, companion animals, vehicles and humans[17–23]. Understanding the spread of LA-MRSA among pig herds is essential to developing meaningful control programs for the pig production sector. The main goal of action plans made by the Danish authorities is to reduce the number of LA-MRSA-positive pigs and herds, thereby reducing the risk of transmission to humans.

Simulation models are widely used as a way to inform decision making. They present an opportunity to test a hypothesis without experiments or in cases where experimental studies are not possible[24,25]. They cover a wide range of applications such as modelling the within-host infection dynamics, disease-spread modelling within or between populations, and evaluating control strategies to support decision makers in the control of infectious diseases[24]. Since EU regulations require identification and registration of animals and animal movements[26], data are available on a large scale and can be used as input in disease-spread

models. Furthermore, if a pathogen is mainly spread through animal movements, the movement data can be used retrospectively to investigate its spread.

An individual-based Monte Carlo simulation model of the spread of LA-MRSA within and between pig herds was developed, integrating available data on Danish pig herds and registered pig movements among these herds. The purpose was to examine what might have influenced the rapid spread of LA-MRSA among Danish pig herds during the period 2006 to 2015. The aims were to: (1) assess whether transmission of LA-MRSA via registered pig movements alone could account for the observed increase in prevalence in Denmark, and (2) evaluate the effect of other transmission mechanisms. The results should assist in creating an agenda for the control of LA-MRSA in Danish pig herds.

MATERIALS AND METHODS

Data background for the simulation model (input data). In Denmark, information on pig holdings and pig movements are registered in the Central Husbandry Register (CHR)[27], which is owned by the Ministry of Environment and Food. A dataset containing information on 18,648 pig herds and 7,678,851 movements among pig herds in Denmark was extracted for the study period 1st January 2006 to 31st December 2015. The dataset is described in detail by Schulz et al. (2017)[13], and data preparation for the simulation model is described in the Supplementary Information. Several herds could be owned by the same farmer and thus constitute one holding. The study was performed at herd level, but information on ownership was retained to aid the modelling of LA-MRSA spread among herds with the same owner.

Herds registered as: (1) breeding and multiplier herds (breeding sites), (2) production herds, organic or free-range pig herds (merged into one category) and weaner herds (production sites), and (3) hobby herds (hobby sites) were used in our analysis, as well as the respective movements among these herds. End-of-production sites (e.g. 133 herds registered as slaughterhouses and 2,961,979 movements to slaughterhouses) were excluded because it was assumed that there would be no spread of pathogens back into the production chain from these. The remaining herd types and respective movements (2,657 herds and 116,386 movement records) were excluded because it was assumed they would have little impact on LA-MRSA spread due to the low number of registered herds and pig movements (e.g. zoo, experimental facilities) or because of missing information on the herd structure and age group of moved pigs (e.g. traders, trade herds). Any movements for which the number of pigs was not recorded were

excluded (5,595 movement records). This left 49 herds that never moved pigs, and these were also excluded. In total, 12,874 herds and 993,474 movements were included in our study. If a herd changed its holding type to one not included in this study, the herd was assumed to be closed for the considered year, but kept active in the model for all other years.

LA-MRSA screening results in Danish pig herds. In 2008, the European Food Safety Authority (EFSA) conducted an LA-MRSA survey in 26 European countries[2]. In subsequent years, national Danish surveys reported a rapid increase in the prevalence of LA-MRSA in breeding herds and slaughter pig herds[11] (Table 1).

Table 1. Overview of LA-MRSA screenings performed in Danish pig herds between 2008 and 2014.

Year[reference]	Description	Number of tested herds	Estimated LA-MRSA prevalence in % [95% confidence interval]
2008[2]	Five dust samples were collected from the immediate environment of breeding pigs in the herd then pooled and tested for LA-MRSA	95 breeding herds: 198 production herds:	0.0 [0.0 – 3.2] 3.5 [1.8 – 7.1]
2010[38]	Pools of five nasal swab samples were taken from five slaughter pigs in five different pens	99 herds with slaughter pigs:	16.2 [9.5 – 24.9]
2011[39]	Pools of five nasal swab samples were taken from five slaughter pigs in five different pens	79 herds with slaughter pigs:	16.5 [9.1 – 26.5]
2014[11]	Pools of five nasal swabs per herd	70 breeding herds: 205 production herds:	63.0 [50.5 – 74.1] 68.0 [60.9 – 74.1]

Simulation model. An individual-based Monte Carlo simulation model for the spread of LA-MRSA among pig herds was developed (Figure 1). Information on pig herds and movement data were read from input files at the beginning of each simulation run. After LA-MRSA was initialised, three processes within each time step were modelled to simulate within-herd dynamics, transmission via pig movements and indirect transmission among herds. For each simulation repetition and on each simulated day, information on positive herds was stored in files for later interpretation.

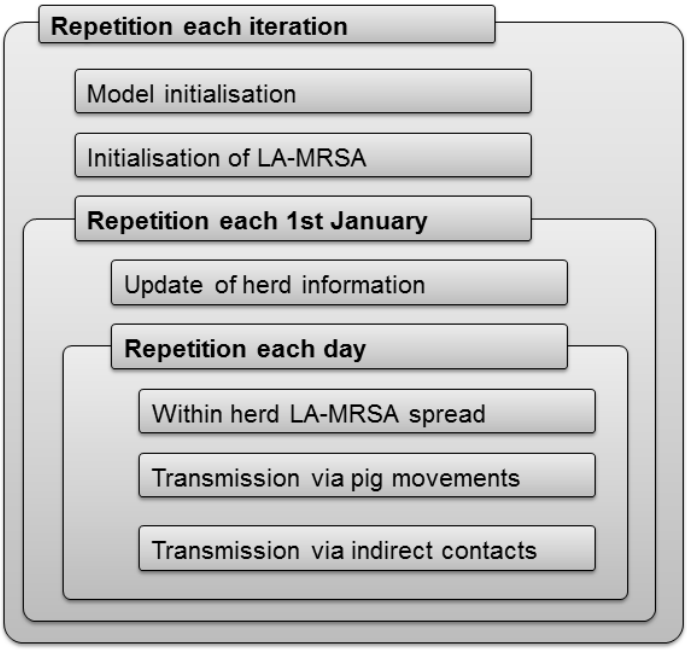

Figure 1. Overview of the structure of the livestock-associated Methicillin-resistant Staphylococcus aureus model.

Model initialisation. In the first step, information on herds and movement data was sourced. Four additional files were read by the model, and these contained: (1) simulation parameters (Table 2); (2) minimum, most likely (mode) and maximum values defining probability distributions (PERT distributions) for transmission and cure rates (Supplementary Information, Table S5); (3) a distance matrix containing the distances between all herds; (4) distance probabilities of contact among herds for the different types of contacts (Supplementary Information, Table S6).

Table 2. Overview of simulation parameters and default values. PERT distributions were defined as transformation of the Beta distribution with minimum (min), maximum (max) and most likely value (mode) and a mean $\mu = \frac{min+4 \cdot mode+max}{6}$.

Variable name	Default value	Description	Reference
Modelling disease spread within a herd			
Environment-related recurrence			
envir	1	Switch on/off environment-related recurrence within-herd (0/1)	
$\boldsymbol{\alpha_{envir}}$	2.5	α in probability function for environment-related recurrence	Within-herd module validation
Within-herd dynamics			
tds	1	Switch on/off time-dependent scaling of within-herd transmission	
$\boldsymbol{x}$	50	Midpoint s-shape transmission curve	Within-herd module validation
$\boldsymbol{k}$	0.05	Steepness of s-shape transmission curve	Within-herd module validation
hm	1	Switch on/off homogeneous mixing in small herds (0/1)	
$\boldsymbol{th_{small}}$	200	Herds sizes below or equal to th_{small} are categorised as small herds – indicating random mixing within herd	Assumption

Variable name	Default value	Description	Reference
Modelling disease spread among herds			
Spread via indirect contacts			
ic	1	Switch on/off transmission via indirect contacts (0/1)	
$\boldsymbol{\lambda_{in}}$	0.256	Average daily probability of indirect contact originating from an LA-MRSA-positive indoor herd	Adjusted based on Boklund et al. (2013)[40]
$\boldsymbol{\lambda_{out}}$	0.1864	Average daily probability of indirect contact originating from an LA-MRSA-positive outdoor herd	Adjusted based on Boklund et al. (2013)[40]
$\boldsymbol{Prob_{in}}$	PERT (min = 0.001, max = 0.01, mode = 0.005071)	Probability of infection via contact from an LA-MRSA-positive indoor herd	Expert opinion
$\boldsymbol{Prob_{out}}$	PERT (min = 0.001, max = 0.01, mode = 0.0035)	Probability of infection via contact from an LA-MRSA-positive outdoor herd	Expert opinion
$\boldsymbol{Prob_{a}}$	PERT (min = 0.001, max = 0.01, mode = 0.004714)	Probability of infection via abattoir movements	Expert opinion

In the next step, a herd matrix was generated containing the following information for each herd: (1) an individual identification number (1 - 12,874); (2) the herd type registered in the CHR in 2006, set to zero for herds that were inactive (i.e. no movements registered) in 2006; (3) the simulated LA-MRSA status (equal to zero); (4) the number of sows, weaners and finishers registered (or estimated numbers as described in the Supplementary Information); (5) the number of LA-MRSA-positive sows, weaners and finishers (equal to zero); (6) status of antimicrobial usage based on the herd information (Supplementary Information); (7) transmission rates; (8) cure rates. Transmission and cure rates were randomly chosen from PERT distributions for each herd based on information from the literature[28] (Supplementary Information, Table S5).

To initialise LA-MRSA in the simulation, it was necessary to define the number of herds and the herd types where LA-MRSA would be seeded. Additionally, the number of LA-MRSA-positive pigs and the age group of the first LA-MRSA cases were set. In the selected herds, ten LA-MRSA-positive sows were initialised. If fewer than ten sows were available, LA-MRSA was initiated in the weaner or finisher section. Several scenarios to mimic different situations in 2006 and different introductions at later points in time were tested (Supplementary Information, Table S7). In 2006, LA-MRSA was seeded exclusively in large herds (number of sows, weaners and finishers above th_{small}), whereas when it was initialised in later years, small herds could also randomly be chosen.

The model simulated daily time steps, while the herd matrix was updated on a yearly basis, e.g. herd type, number of animals and antimicrobial usage were updated according to the input data. Transmission and cure rates were only updated if the herd type changed between two consecutive years. The number of positive animals in each age group was calculated for the next year based on the prevalence in the previous year. If the herd type changed between years, the LA-MRSA status and number of positive sows, weaners and finishers was set to zero, with the assumption that cleaning and disinfection were carried out before changing the herd type.

Modelling disease spread within a herd

The following processes were implemented and used in the order they are described:

(1) *Environment-related recurrence.* For herds that had previously been LA-MRSA positive but had no LA-MRSA-positive pigs on the simulation day (due to self-cleaning or selling all positive pigs), the LA-MRSA status of the herd was set to negative in the model. Nevertheless, depending on the time since

the status changed to negative, an exponentially decreasing probability of one pig reverting to LA-MRSA positive status due to environmental contamination was simulated (Supplementary Information).

If the herd type changed between two consecutive years, this recurrence due to environmental contamination was still possible.

(2) *Self-cleaning of individual pigs.* In positive herds, pigs could clear themselves randomly at any time according to the cure rates for sows, weaners and finishers within the herd (Supplementary Information, Table S5).

(3) *Within-herd dynamics.* A three-compartment within-herd model was implemented with transmission within the three compartments and high- and low-risk transmission routes between the compartments for sows, weaners and finishers (Figure 2). Homogenous mixing within each compartment was assumed. High-risk transmission was assumed between subsequent compartments within the production line to mimic LA-MRSA transmission due to pig movements within the herd. A lower risk of LA-MRSA transmission was assumed in the opposite direction to the production line, representing indirect transmission mechanisms e.g. via humans or equipment.

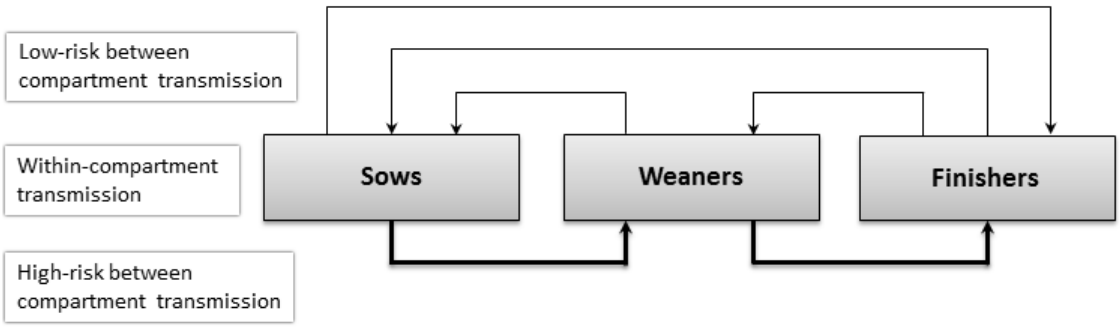

Figure 2. Schematic description of the implemented within-herd LA-MRSA spread.

The transmission parameters published by Broens et al.[28] (Supplementary Information, Table S5) take into account the pen structure in the compartments, which was not reflected in our model. The development of the within-herd prevalence in the first year after initialisation of LA-MRSA therefore showed a rapid increase (Supplementary Information, Figures S1-S4). In order to slow down the within-herd spread to reflect pen structure, a time-dependent shifting function was introduced (as described in Equation 5 in the Supplementary Information), representing the speed and steepness of LA-MRSA spread as modelled by Sørensen et al.[29]

In herds with fewer than a certain number of animals (default value: $\mathrm{th}_{\mathrm{small}} = 200$), only one compartment was modelled and homogeneous mixing was assumed. No time-dependent shifting was used for the transmission rates in small herds. Supplementary Information Figure S5 shows the within-herd prevalence in production herds with fewer than 200 animals for (a) transmission rates adapted from Broens et al.[28] and (b) time-shifted transmission rates for (1) herds using high-risk antibiotics, and (2) herds not using high-risk antibiotics.

All subsequent scenarios were run with time-shifted transmission rates adapted based on Broens et al.[28] and Sørensen et al.[29]

Modelling disease spread among herds

Spread via animal movements. Danish movement data do not include information on the age group of pigs moved between herds. As differences in prevalence can be expected among age groups, it was necessary to define movement types reflecting the age groups for each movement. The movement types were based on the number of registered sows, weaners and finishers in both the sending and receiving herd (Supplementary Information).

Transmission via pig movements was included based on the movement data and types. The number of LA-MRSA-positive pigs that were moved to the receiving herd was calculated using a binomial process representing the total number of pigs that were moved and the prevalence within the source compartment. The herd size in both sending and receiving herds was kept constant, with the assumption that whenever pigs were moved in, a similar number of pigs were moved out. However, the number of LA-MRSA-positive pigs was updated on a daily basis for both herds. If there were no weaners registered in the sending herd, but the age group of pigs moved out of this herd was estimated to be weaners, the

prevalence in the sow section was used. Nevertheless, the number of positive sows in this section was not updated.

Spread via indirect contacts. Other transmission routes such as vehicles (feeding and abattoir trucks) and humans (veterinarians, technicians and visitors) were pooled as indirect contacts among herds.

Two pathways of indirect transmission were modelled: (1) distance-dependent transmission from LA-MRSA-positive herds via e.g. humans moving between farms, and (2) transmission related to abattoir movements. Indirect transmission related to human and abattoir movements was restricted to contacts within the same day of visits to several herds. For each LA-MRSA-positive herd h, the number of indirect contacts was calculated based on a Poisson distribution using λ_{in}, λ_{out}, or λ_a^h . It was determined whether each indirect contact was effective based on the PERT distributions $Prob_{in}$, $Prob_{out}$ or $Prob_a$ given in the input file (Table 2). In the case of an effective indirect contact, a herd was randomly chosen based on the read-in distance probability tables (Supplementary Information, Table S6).

In addition, transmission among herds with the same owner was modelled to represent shared workers or equipment (Supplementary Information).

Validation and convergence. The model was internally validated using three methods[30]: (1) face validity – using flow charts to ask people with an insight into the system whether the conceptual model was reasonable (Figure 1, Figures S4-S8), and in the case of within-herd validation, whether the within-compartment prevalence represented a meaningful range, (2) the tracing method - following individual herds over time to determine whether the logic was correct, and (3) sensitivity analysis - evaluating the effect of changes in the fixed parameters. In addition, external validation was conducted by comparing the predicted prevalence of LA-MRSA by the model to the observed prevalence of LA-MRSA from the screening results in Danish swine herds (Table 1).

We assessed the number of simulation repetitions required before the variance in the total number of LA-MRSA-positive herds stabilised on 31st December 2015 (Supplementary Information, Figure S9). Although convergence was reached after 250 iterations, the model was run with 500 iterations per scenario to cover any extra variability in the different initialisation scenarios and in the sensitivity analysis.

A sensitivity analysis was performed based on a scenario in which LA-MRSA was initialised in 100 active large production herds on 1st January 2006, as well as one breeding and multiplier herd and 100 production herds on 1st January 2009. The model was run with different settings (e.g. without

environment-related recurrence, Table 3) and all fixed parameters were halved and doubled (Table 3). The effects of changes were evaluated by changes in the within-herd dynamics and the total number of infected herds 10 years after the first initialisation of LA-MRSA.

Data processing, simulation modelling and graphical presentation of results were performed in R version 3.2.2 - "Fire Safety"[31].

Table 3. Overview of scenarios run in the framework of the sensitivity analysis. All default values (Table 2) were halved and doubled. For PERT distributions ($Prob_{in}$, $Prob_{out}$, $Prob_a$), minimum, mode and maximum values were halved and doubled.

Acronym	Description	Values
Modelling disease spread within a herd		
Environment-related recurrence		
NoEnvir	No environment-related recurrence within-herd	$envir = 0$
Envir	Variation of α_{envir}	$\alpha_{envir} \in \{1.25, 5\}$
Within-herd dynamics		
NoTDS	No time-dependent shifting of within-herd transmission	$tds = 0$
Mid	Variation of *x*	$x \in \{25, 75\}$
Steep	Variation of *k*	$k \in \{0.025, 0.1\}$
NoHM	No homogeneous mixing in small herds	$hm = 0$
THhm	Variation of th_{small}	$th_{small} \in \{100, 400\}$
Modelling disease spread among herds		
Spread via indirect contacts		
NoIC	No indirect contacts	$ic = 0$
$IC_{in/out}$	Only distance-dependent indirect contact for indoor and outdoor herds	
IC_a	Only indirect contact related to abattoir movements	
IC_{sw}	Only indirect contact related to herds with the same owner	
$FreqIC_{in/out}$	Variation of $\lambda_i$and λ_o	$\lambda_i \in \{0.128, 0.512\}$ $\lambda_o \in \{0.0932, 0.3728\}$
$ProbIC_{in/out}$	Variation of $Prob_{in}$ and $Prob_{out}$	$Prob_{in} \in \{PERT(0.0005, 0.005, 0.0025355),$ $PERT(0.002, 0.02, 0.010142)\}$ $Prob_{out} \in \{PERT(0.0005, 0.005, 0.00175),$ $PERT(0.002, 0.02, 0.007)\}$
$ProbIC_a$	Variation of $Prob_a$	$Prob_a \in \{PERT(0.0005, 0.005, 0.002357),$ $PERT(0.002, 0.02, 0.009428)\}$

RESULTS

Animal movements alone (initialisation scenarios). To assess whether the transmission of LA-MRSA via pig movements alone could account for the observed pattern of increased prevalence in Denmark, the model was run with 17 initialisation scenarios (Supplementary Information, Table S7). Based on the assumption that LA-MRSA was already established in 2006, five scenarios were set up which introduced LA-MRSA in varying quantities of breeding and multiplier and/or production herds in 2006. Additionally, in 12 scenarios, LA-MRSA was introduced at later points in time to mimic new introductions. The simulated proportion of LA-MRSA-positive holdings in 2008 and 2014 was compared to the observed results of LA-MRSA screenings performed in Denmark (Table 1). In all scenarios, LA-MRSA was initialised on 1st January 2006 in a number of breeding and multiplier herds and/or production herds (Supplementary Information, Table S7). Furthermore, scenarios were initiated to mimic further introduction of LA-MRSA in subsequent years. The predicted prevalence in breeding and multiplier herds matched the results of LA-MRSA screenings performed in Danish pig herds in 2008 (Table 1), but was lower than the observed prevalence in 2014 (Figure 3). Animal movements could explain the observed prevalence in production herds in 2008 for certain scenarios, for example when the spread was initiated in many herds in 2006, or combined with initialisation in breeding herds. However, none of the scenarios could explain the observed prevalence in 2014 using animal movements alone (Figure 3 and Supplementary Information, Figure S6).

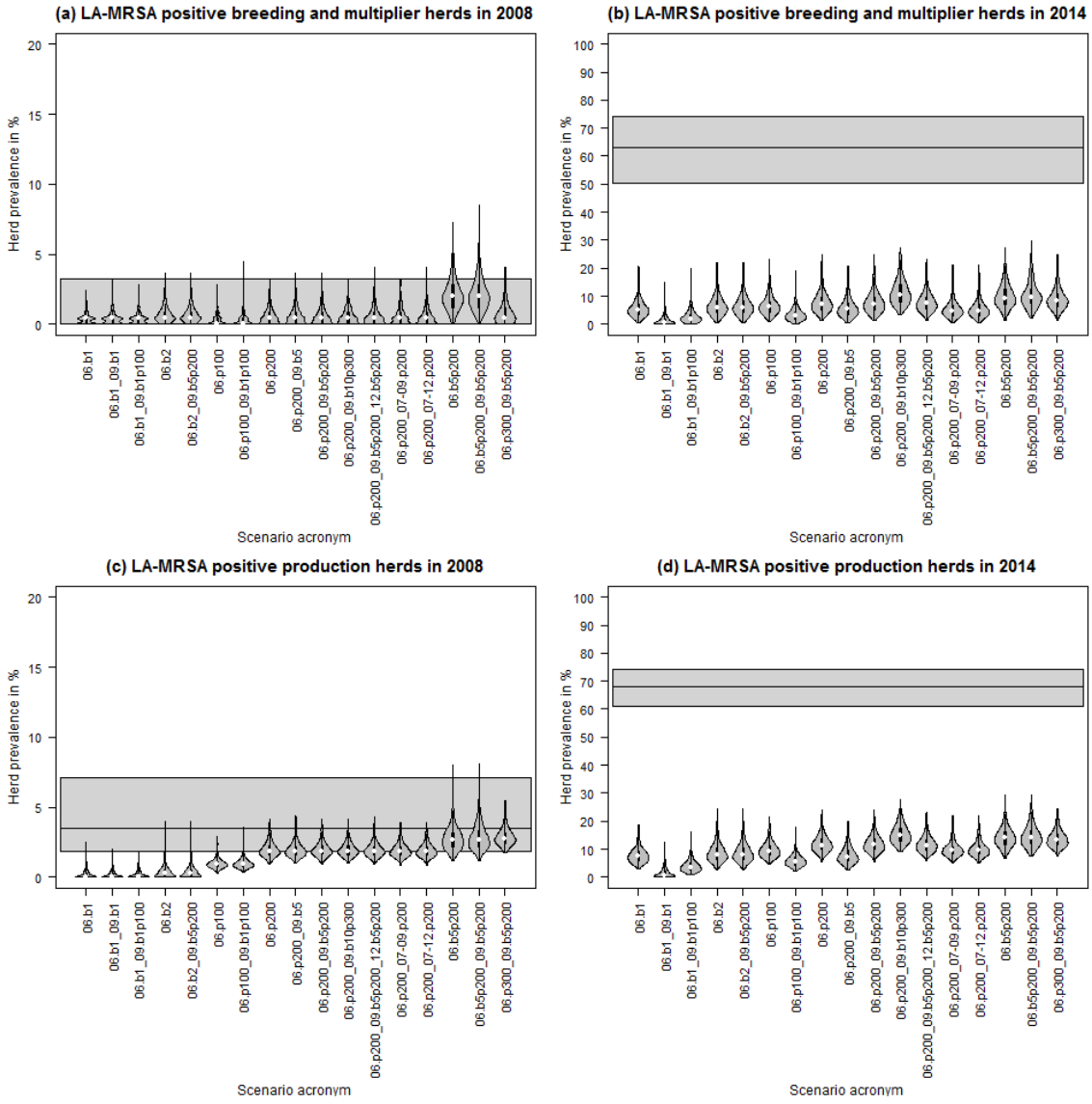

Figure 3. Herd prevalence in breeding and multiplier herds (a+b) and production herds (c+d) on 31st December 2008 and 31st December 2014 resulting from simulated LA-MRSA transmission via pig movements only, based on 500 iterations. Time-shifted transmission rates adapted from Broens et al.28 were used. The horizontal black line and grey area mark the observed prevalence and 95% confidence interval of the LA-MRSA screenings performed in Danish swine herds in 2008 and 2014. Example acronyms: 06.b5p200 – initialisation in five breeding and multiplier herds and 200 production herds in 2006, 06.b5_07-09.p200 – initialisation in five breeding and multiplier herds in 2006 and 200 production herds each year from 2007 to 2009.

Animal movements and indirect contacts (initialisation scenarios). In the next step, all initialisation scenarios were run using pig movements and indirect contacts as transmission mechanisms and the between-herd prevalence on 31st December 2015 was compared. In all scenarios, the modelled

prevalence was higher when indirect contacts were added, compared to simulations in which transmission was modelled via animal movements alone (data not shown).

In seven of the initialisation scenarios, the simulated median prevalence in breeding and multiplier herds as well as in production herds overlapped with the confidence interval of the LA-MRSA screening results for Danish pig herds in 2008 (Figure 4). The median of five of the mentioned scenarios overlapped with the 2014 screening results for breeding and multiplier herds (Figure 4b). However, for production herds, the median of only one scenario overlapped with the 2014 LA-MRSA screening results, whereas the median prevalence in 2008 exceeded the observed screening results (Figure 4). The violin plots overlapped with the screening results for breeding and multiplier herds as well as for production herds in both 2008 and 2014 in eight initialisation scenarios, indicating that these scenarios might represent the spread of LA-MRSA among pig herds between 2006 and 2014.

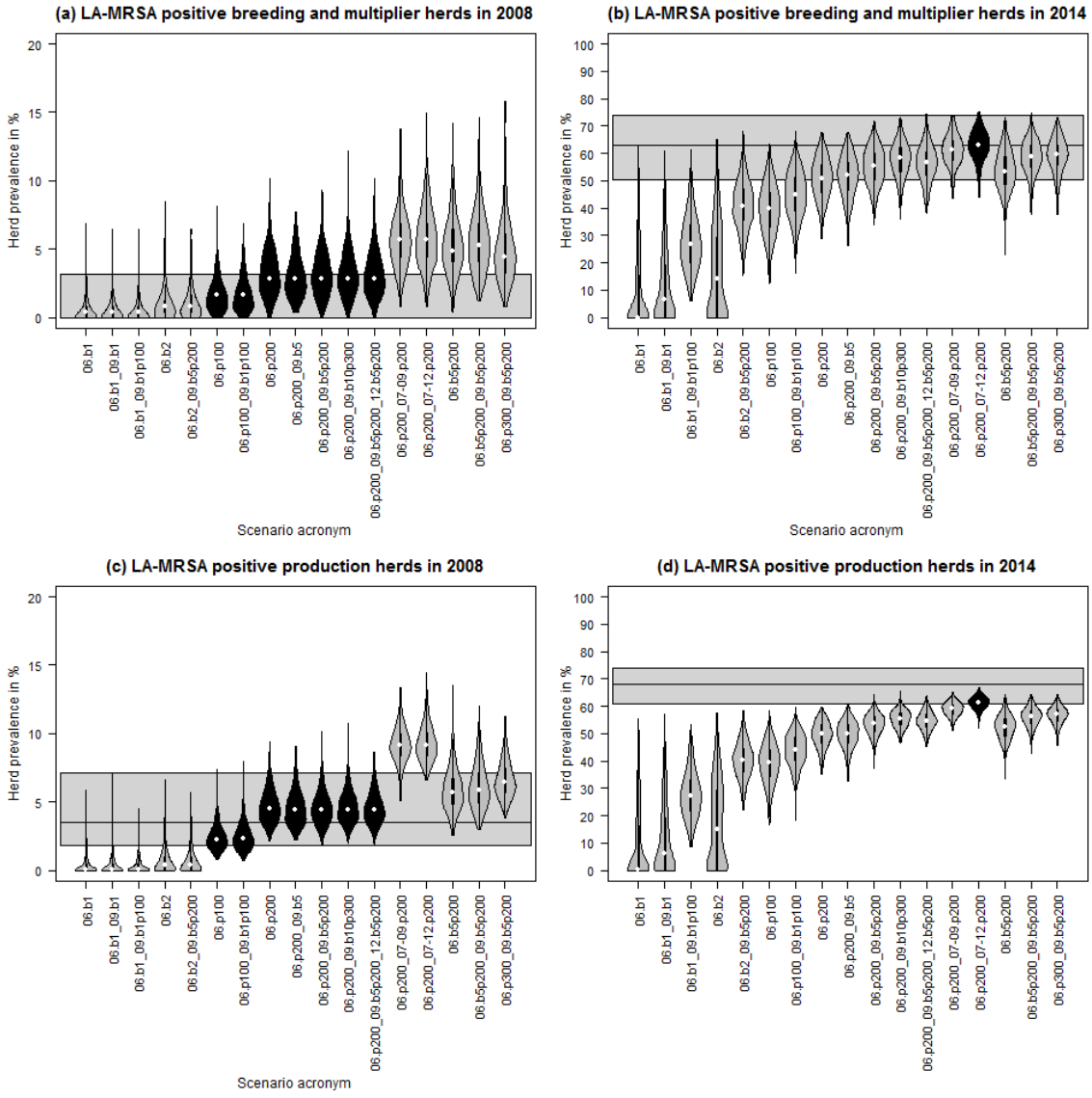

Figure 4. Herd prevalence in breeding and multiplier herds (a+b) and production herds (c+d) on 31st December 2008 and 31st December 2014 resulting from simulated LA-MRSA transmission via pig movements and indirect contacts, based on 500 iterations. Time-shifted transmission rates adapted from Broens et al.[28] were used. The horizontal black line and grey area mark the observed prevalence and 95% confidence interval of the LA-MRSA screenings performed in Danish swine herds in 2008 and 2014. Black violins mark scenarios in which the simulated median prevalence in breeding and multiplier herds and production herds overlapped with the confidence interval of the LA-MRSA screening results. Example acronyms: **06.b5p200** – initialisation in five breeding and multiplier herds and 200 production herds in 2006, **06.b5_07-09.p200** – initialisation in five breeding and multiplier herds in 2006 and 200 production herds each year from 2007 to 2009.

Sensitivity analysis. Based on the results, a default scenario of LA-MRSA initialisation in 100 production herds in 2006, and in one breeding and multiplier and 100 production herds in 2009 was used for the sensitivity analysis. The results of the scenarios with varied parameters were compared to the predicted median prevalence of the default scenario to allow the evaluation of the effects of parameter variation.

Scenarios relating to within-herd dynamics had a large impact on the model outcome. Running the model without time-dependent scaling of the transmission rates (NoTDS), and with halved midpoint (Mid_h) or doubled steepness (Steep_d) of the time-scaling function resulted in a higher prevalence compared to the standard scenario (Figure 5). The modelled prevalence in the scenarios Mid_h and Steep_d fitted the LA-MRSA screening results from 2014 better than the default scenario. However, the modelled results for 2008 were above the LA-MRSA screening results. Compared to the default scenario, reducing the within-herd transmission by doubling the midpoint or halving the steepness of the time-scaling function decreased the modelled prevalence for breeding and multiplier herds and production herds for both years.

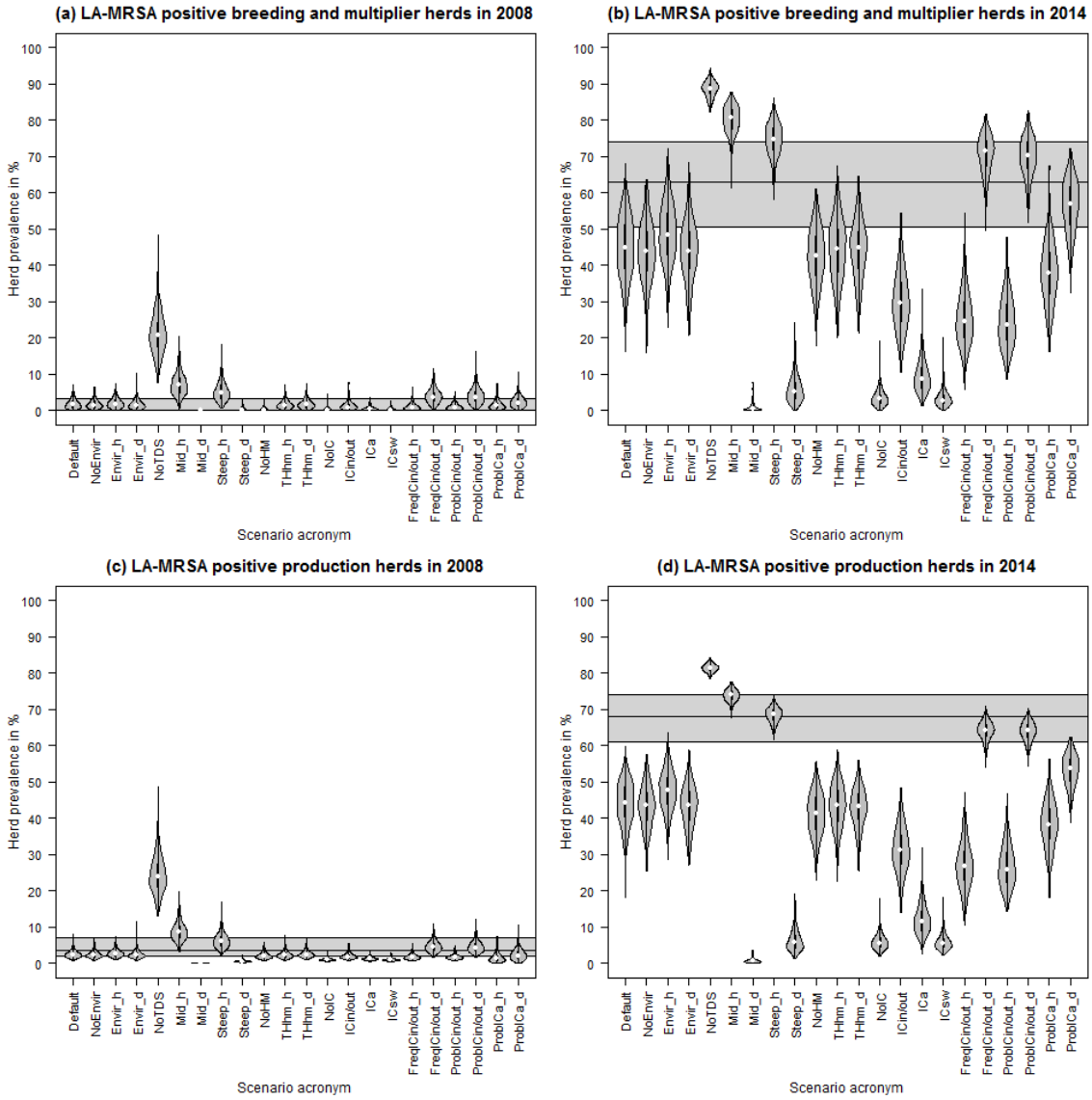

Figure 5. Sensitivity analysis showing the effect of changing the input for herd prevalence in breeding and multiplier herds (a+b) and production herds (c+d). Based on prevalence on 31st December 2008 and 31st December 2014 in 500 iterations. Time-shifted transmission rates adapted from Broens et al.[28] were used. The horizontal black line and grey area mark the observed prevalence and 95% confidence interval of the LA-MRSA screenings performed in Danish swine herds in 2008 and 2014.

Scenarios representing environment-related recurrence (NoEnvir, Envir_h, Envir_d) and homogeneous mixing in small herds (NoHM, THhm_h, THhm_d) showed only small variations in the modelled outcome compared to the default scenario (Figure 5).

Simulations that only modelled one of the implemented indirect transmission routes also resulted in a lower prevalence compared to the default scenario. Nevertheless, doubling (1) the average daily probability of indirect contact originating from an LA-MRSA-positive herd (variables λ_{in} and λ_{out}, scenario FreqIC$_{in/out}$_d) or (2) the probability of infection via indirect contact from an LA-MRSA-positive herd (variables Prob$_{in}$ and Prob$_{out}$, scenario ProbIC$_{in/out}$_d) led to an increased simulated prevalence in 2008 and 2014 compared to the default scenario, as well as an overlap with the LA-MRSA screening results from 2014.

Violin plots for 2010 and 2011 are shown in Supplementary Information, Figure S8.

DISCUSSION

Livestock movements have been described as a driving factor in the spread of LA-MRSA, and the restriction of trade from MRSA-positive to MRSA-negative herds has therefore been discussed in the literature as a potential control option[32]. In order to assess whether a ban on animal movements would impede LA-MRSA spread, it is important to understand the extent to which it affects transmission. In the present study, the spread of LA-MRSA among pig herds via animal movements alone and via animal movements combined with indirect contact among pig herds was simulated to evaluate the effect of the modelled transmission routes. Several initialisation and sensitivity analysis scenarios were run and the results compared to the outcome of LA-MRSA screenings performed in Danish pig herds to externally validate the model and to evaluate the influence of the used model parameters on the model prediction. Based on assumptions in the model, pig movements alone cannot account for the spread of LA-MRSA in Denmark for the period 2006 to 2014. Even extensive initialisation in 2006 and subsequent years did not lead to the increase in LA-MRSA prevalence observed in Danish pig herds. Therefore, it is likely that a prevention strategy including only a ban on animal movements would not have prevented the spread of LA-MRSA among Danish pig herds.

Three routes were included to mimic the transmission of LA-MRSA via indirect contact: (1) human contacts, (2) abattoir movements, and (3) same owner. Modelling indirect contact clearly showed that the model was able to represent the observed prevalence in 2008, but showed lower predicted prevalence compared to that observed in 2014 for the default scenario (Figure 4). Still, the figure clearly demonstrates the importance of indirect contact in the spread of LA-MRSA among pig herds compared to

modelling the spread using only animal movements (Figures 3-4). The sensitivity analysis on the frequency and risk associated with indirect contact also showed its influential impact on the spread of LA-MRSA (Figure 5). An LA-MRSA control program must therefore include biosecurity measures to limit the spread of LA-MRSA among herds via indirect contact. However, even though literature describing these transmission routes does exist[21,33,34], detailed information on the frequency and effectiveness of LA-MRSA transmission via these indirect routes is lacking. As parameters related to indirect transmission routes were shown to be relevant for LA-MRSA spread among pig holdings, more knowledge should be gained to reduce uncertainties in the model outcome.

The model was initiated with many scenarios in an attempt to generate situations that would mimic the observed prevalence. The scenarios in which LA-MRSA was only initiated in herds in 2006 did not mimic the observed prevalence (Figure 4). Nevertheless, randomly infecting herds in subsequent years resulted in an overlap between the some of the scenarios and the observed prevalence, indicating that the model is capable of replicating the LA-MRSA epidemic in Denmark between 2006 and 2014. As it is not possible to know exactly which (if any) of the modelled scenarios corresponded to reality, it can be difficult to choose the best scenario for future modelling of control measures. However, several scenarios can be modelled to identify the most effective strategy in different situations.

The fact that LA-MRSA had to be initialised in many herds over time suggests that it could not have spread solely via the simulated processes in this study (namely animal movements and indirect contacts), but that other processes must also have contributed. Humans can be persistent carriers of *S. aureus* and therefore be colonised for longer time periods[35]. In addition, humans working at or visiting a known LA-MRSA-positive herd might carry the pathogen for some time[36] and might be able to transmit it to pigs in other visited herds. Therefore, the unexplained introduction of LA-MRSA to pig herds over time could represent introductions via humans. LA-MRSA could have been spread locally via for example, air, companion animals or rodents. However, to the best of our knowledge, there are no studies providing quantification of LA-MRSA transmission through these routes, and they were therefore not incorporated into the model framework.

Results of the sensitivity analysis showed that the parameters representing the within-herd spread of LA-MRSA had a substantial impact on model predictions (Figure 5). This indicated that the within-herd

prevalence could affect the between-herd prevalence and that reducing the prevalence within a herd might help to control LA-MRSA spread. This can be used as a starting point to study the impact of reducing the within-herd prevalence, for example by reducing antibiotic usage, and/or imposing higher biosecurity in a national control program.

Real data on pig herds and movements registered in the CHR were used as background data for the simulation[27]. Although the CHR provides very detailed data, it was necessary to estimate the age groups of moved pigs and, in some cases, the number of animals in a certain age group on the farm. Obligatory registration of the age of moved pigs in the CHR and automatic cross-validation between registers would increase the accuracy and validity of data in general as well as the accuracy of the results from this study.

It was necessary to exclude all trade herds due to missing information on the structure of the trade facilities. As there is no individual identification system for pigs, it was impossible to identify how long pigs might have been owned by traders or how they were mixed, thus potentially spreading LA-MRSA. However, there are very few trade herds in Denmark[13] and this was therefore considered to be of limited importance.

Only one study simulating the spread of LA-MRSA among pig herds was found in the literature[39]. This study was conducted on pig herds in Denmark and it found that movement-induced transmission alone could yield a high probability of LA-MRSA persistence at a very low prevalence. However, the study used restricted movement data from only 1 year and did not include within-herd dynamics, which appeared to be influential in our study. In addition, indirect contact was modelled implicitly, without modelling the frequency or associated risk, and the impact of indirect contact on LA-MRSA spread was therefore not studied.

Besides individual-based Monte Carlo simulation models, also population-based models exist[25,37]. However, these population-based models simulate groups of individuals (herds), and actions such as pig movements or indirect contacts between herds would be applied to all herds in a certain group at the same time, which is not realistic. In contrast, individual-based models allow tracing of individual herds over time providing better insight into the modelled processes.

The model was developed to gain better insight into the spread of LA-MRSA among pig herds. However, it could be adapted to mimic the transmission of other pathogens that can be shed without clinical signs in pigs such as *Salmonella*. . These pathogens could also be transmitted via animal movements and indirect contacts among pig herds. In addition, the model can also be used to simulate the spread of LA-MRSA in other farmed animal species. When applying the model to other pathogens or animal species, it may require adaptation according to the modelled disease or system. Modules mimicking other transmission pathways than the already implemented could be easily added to the original model.

In future, this model can be used to test control options to limit the spread of LA-MRSA. The effects of e.g. trade restrictions, as well as constraints related to indirect contact among herds could be evaluated to assist decision makers adapt local action plans. Additionally, the impact of increasing internal biosecurity and attempts to reduce antimicrobial usage at herd level could be evaluated. This could lead to the establishment of potential thresholds below which the within-herd prevalence would have to remain in order to have a clear impact on the between-herd spread of LA-MRSA.

Data Availability. Data are owned by a third party. The authors had no special access privileges to these data. Danish pig movement data can be obtained for a fee by ordering an extract from https://chr.fvst.dk and by contacting the CGI Service Desk, tel. +45 70 21 13 21 or e-mailing dk-support.dk@cgi.com.

Code availability. The R script can be obtained from the authors upon request.

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ADDITIONAL INFORMATION

Author contributions. N.T., T.H. and A.B. initiated the project; J.S. performed the simulations and calculations; A.B. and T.H. supervised the project; all authors interpreted the results and produced the final manuscript.

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3.3. MANUSCRIPT III: EFFECTS OF CONTROL MEASURES ON THE SPREAD OF LA-MRSA AMONG DANISH PIG HERDS BETWEEN 2006 AND 2015 - A SIMULATION STUDY

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Effects of control measures on the spread of LA-MRSA among Danish pig herds between 2006 and 2015 – a simulation study

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ABSTRACT

There has been a rapid increase in Danish pig herds testing positive for livestock-associated Methicillin-resistant *Staphylococcus aureus* (LA-MRSA) since the first screening in 2008. Despite a national action plan to control LA-MRSA in the Danish pig population, 88% of pig herds tested positive in a 2016 cross-sectional study of 57 herds. The national action plan was initiated in April 2015 and aimed to reduce the spread of LA-MRSA among pig herds. However, its success is uncertain. We used a simulation model mimicking the spread of LA-MRSA among pig herds between 2006 and 2015 to evaluate the impact of control strategies if these had these been implemented in 2007 or 2010. The strategies were combinations of the following control measures: (1) a reduced number of herds using high-risk antibiotics, (2) a reduced probability of indirect transmission among herds via humans, (3) movement restrictions, and (4) voluntary eradication in 5-7.5% of the herds. Almost all tested control strategies simulated a reduction in the spread of LA-MRSA. The combination of two, three or four intervention strategies showed additive effects and led to larger reductions in the predicted herd prevalence. In addition, the prevalence of LA-MRSA-positive herds at the time when control measures were initiated influenced the effects of the control strategies. Combining the simulated control measures can be considered in future action plans to control LA-MRSA.

KEYWORDS

Simulation model, LA-MRSA, Denmark, pig movements, swine, control

INTRODUCTION

Methicillin-resistant *Staphylococcus (S.) aureus* (MRSA) is a group of *S. aureus* that is resistant to most β-lactam antibiotics[1]. The main reservoir for livestock-associated MRSA (LA-MRSA) is the pig population, though it has also been found in humans and other animal species[2]. In humans, (LA-) MRSA can cause severe infections in children, elderly or immunosuppressed people. The number of LA-MRSA cases in humans has increased in recent years[3]. Transmission from livestock to humans has been established[4,5], so limiting the spread of LA-MRSA in the pig population may limit the number of LA-MRSA cases in humans.

The first detection of LA-MRSA in Danish pig farms was in isolates from samples taken in 2006[6]. In 2008, a survey conducted in 26 European countries by the European Food Safety Authority (EFSA) found 3% of Danish production herds, but no Danish breeding herds, were positive for LA-MRSA type CC398[7]. However, a 2014 national survey found a prevalence of 63% in breeding herds and 68% in slaughter pig herds[8], and a similar survey from 2016 found a prevalence of 88% in finisher herds[3].

Danish pig production has a pyramidal structure with breeding herds at the top, production herds in the middle and slaughterhouses at the bottom[9]. Pig movements mainly occur vertically in accordance with pig production, but horizontal connections also exist[9]. Pig movements were identified as an important route for the spread of LA-MRSA among pig herds[10,11]. The Danish Veterinary and Food Administration (DVFA) published an action plan for controlling LA-MRSA in April 2015, based on recommendations from a risk assessment[12]. This action plan aimed to reduce the use of antibiotics in pig production by 15% from 2015 to 2018, thereby reducing levels of LA-MRSA in pig herds. Catry et al. (2010)[13] described potential control measures to limit the spread of LA-MRSA among pig herds based on risk factors for LA-MRSA spread. They suggested: (1) improved hygiene within herds and during transport, beginning with the breeding herds and followed by the rest of the production chain, and (2) prevention of pig movements from MRSA-positive to MRSA-negative herds.

Schulz et al. (2018)[14] developed an agent-based Monte Carlo simulation model of the spread of LA-MRSA among pig herds in order to study the epidemic behaviour and to identify the driving factors in LA-MRSA spread among pig herds. The model suggested that the spread of LA-MRSA could be explained by three transmission routes: animal movements, indirect contact and unexplained introductions. None of the three transmission routes on their own were able explain the rapid increase in LA-MRSA prevalence in Denmark. However, combining all three routes under the model assumptions mimicked a development of

LA-MRSA-positive herds similar to the trend observed in Denmark. Both the frequency and effectiveness of indirect contact with humans visiting more than one herd on the same day were identified as sensitive parameters in the model presented by Schulz et al.[14] The model can be used to assess which control measures could have been used to control the spread of LA-MRSA among herds. Retrospective studies on how an epidemic in a country/region could have had been influenced may aid in controlling future epidemics in the same or in similar areas.

The objective of this study was to investigate how the spread of LA-MRSA among Danish pig herds between 2006 and 2015 could have been influenced by: (1) a reduced number of herds using high-risk antibiotics, (2) a reduced probability of indirect transmission via humans visiting more than one herd per day (reflecting high levels of biosecurity), (3) movement restrictions between LA-MRSA-positive and negative herds, and (4) voluntary eradication of MRSA in 5-7.5% of the herds. Additionally, we compared two starting points for these control actions to evaluate the impact on the reduction of LA-MRSA spread.

MATERIALS AND METHODS

Simulation model

We used an agent-based Monte Carlo simulation model mimicking the spread of LA-MRSA among Danish pig herds between 2006 and 2015. Figure 1 illustrates the structure of the original model, which is described in detail by Schulz et al.[14] Herd information on Danish pig herds and movement data from 1st January 2006 to 31st December 2015 were used as the basis for modelling.

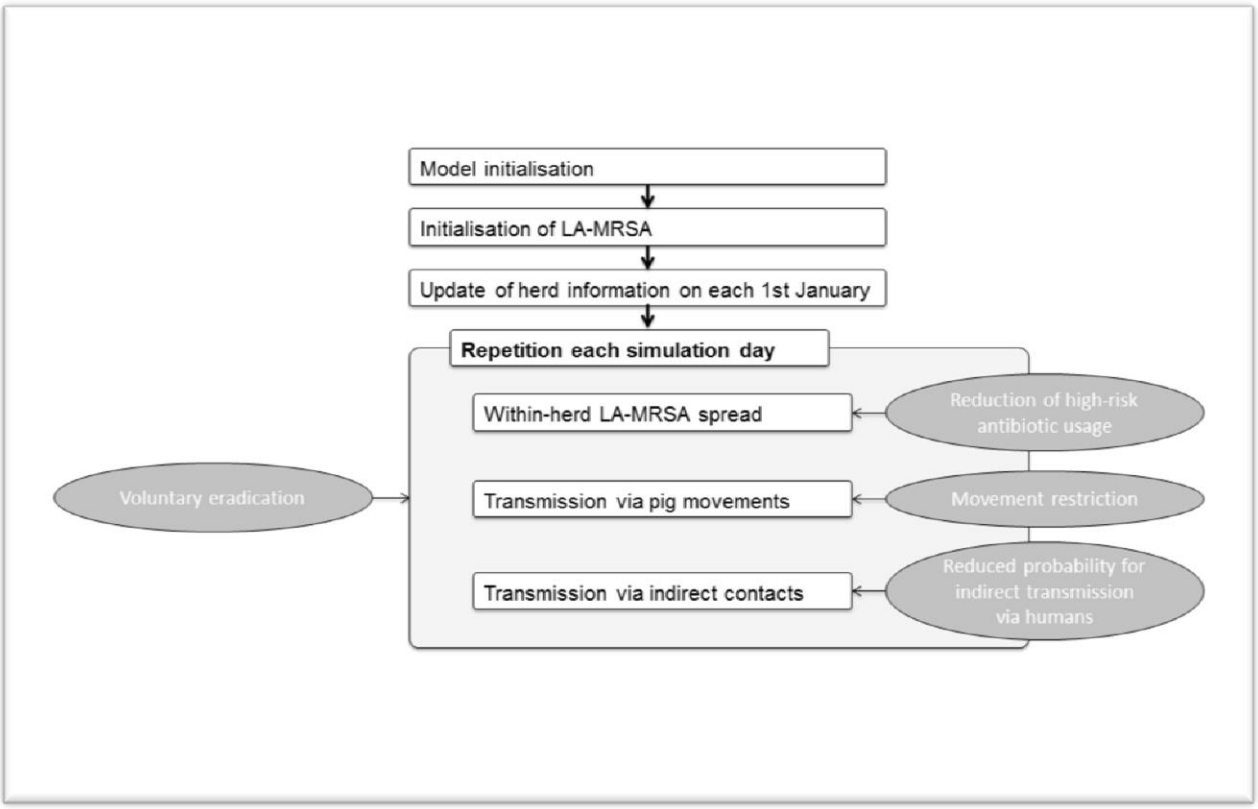

Figure 1. Structure of the LA-MRSA spread simulation model (adapted from Schulz et al.14). The original structure is enhanced by four potential control strategies (dark grey ellipses).

Within-herd spread was simulated using a three-compartment SIS model with different transmission rates within the three compartments of sows, weaners and finishers, and with high- and low-risk transmission routes between these compartments (Table 1). PERT distributions with higher minimum, mode and maximum values were used for herds using high-risk antibiotics. A stochastic and time-discrete simulation process was implemented to mimic spontaneous recovery from LA-MRSA (i.e. colonised pigs could randomly be cleared of LA-MRSA at any time, according to pre-defined cure rates).

Table 1. Assumed values for a PERT distribution to define herd-specific transmission rates based on the use of high-risk antibiotics, adapted by Broens et al.15 The original table was presented in Schulz et al.14

Use of high-risk antibiotics	Within-compartment transmission rate	Low-risk between-compartment transmission rate	High-risk between-compartment transmission rate
no	min = 0.111	min = 0.00175	min = 0.07184
	max = 0.856	max = 0.00301	max = 0.48155
	mode = 0.307	mode = 0.00233	mode = 0.18301
yes	min = 0.211	min = 0.00330	min = 0.13689
	max = 2.924	max = 0.01029	max = 1.64515
	mode = 0.784	mode = 0.00583	mode = 0.46796

Between-herd spread was modelled via two routes: direct and indirect contact among pig herds. Direct transmissions were modelled using data on animal movements registered in the Central Husbandry Register (CHR)[16]. Registered movements among herds were used directly in the model. In the original model, the number of registered sows, weaners and finishers and the number of positive pigs in each of these compartments was recorded for each herd during the simulation. Each pig movement record consisted of the date of the movement, the number of pigs moved (batch size) and the types of pigs moved out of the sending herd and into the receiving herd (i.e. sows, weaners or finishers could be moved out of the sending herd and sows, weaners or finishers could be moved into the receiving herd). Pig movements were modelled as follows:

(1) If the sending herd was negative for LA-MRSA (i.e. the number of positive pigs was zero in each of the three compartments), no pigs were moved because transmission was not possible, and herd sizes were kept constant during each simulated year.

(2) If the sending herd was LA-MRSA positive, the number of LA-MRSA-positive pigs in the movement batch was calculated based on the prevalence of the sending herd (i.e. if the sending herd had a within-herd prevalence of 50%, the prevalence in the movement batch was also assumed to be 50%). In the receiving herd, an increased prevalence was calculated based on the number of positive pigs in the receiving herd plus the number of positive pigs in the movement batch.

Indirect contact was mimicked as the transmission of LA-MRSA via humans visiting more than one pig herd per day and via trucks that collect pigs for slaughter from more than one herd on the same day. While data for the collection of pigs sent to the abattoir was available in the movement register and these were used to calculate herd-specific lambdas, herd-level data did not exist for visitors. This contact type was modelled as a Poisson distribution with the same mean (λ) for all indoor or outdoor herds (Table 2). The probability of infection via indirect contact with LA-MRSA-positive herds was modelled as a PERT distribution (Table 3).

Table 2. Overview of simulation parameters and default values used in the LA-MRSA spread model developed by Schulz et al.[14] Only those parameters related to transmission via indirect contact that varied in the presented study are shown.

Variable name	Default value	Description	Reference
Modelling disease spread among herds			
Spread via indirect contact			
$\boldsymbol{\lambda_{in}}$	0.256	Average daily probability of indirect contact originating from an LA-MRSA-positive indoor herd	Adjusted based on Boklund et al. (2013)[17]
$\boldsymbol{\lambda_{out}}$	0.1864	Average daily probability of indirect contact originating from an LA-MRSA-positive outdoor herd	Adjusted based on Boklund et al. (2013)[17]
$\boldsymbol{Prob_{in}}$	PERT (min = 0.001, max = 0.01, mode = 0.005071)	Probability of infection via contact from an LA-MRSA-positive indoor herd	Expert opinion
$\boldsymbol{Prob_{out}}$	PERT (min = 0.001, max = 0.01, mode = 0.0035)	Probability of infection via contact from an LA-MRSA-positive outdoor herd	Expert opinion
$\boldsymbol{Prob_{a}}$	PERT (min = 0.001, max = 0.01, mode = 0.004714)	Probability of infection via abattoir movements	Expert opinion

Table 3. Simulation parameters related to the probability of infection via contact from LA-MRSA-positive indoor and outdoor herds used in Schulz et al.[14]

Variable name	Default value	Description
$Prob_{in}$	PERT (min = 0.001, max = 0.01, mode = 0.005071)	Probability of infection via contact from an LA-MRSA-positive indoor herd
$Prob_{out}$	PERT (min = 0.001, max = 0.01, mode = 0.0035)	Probability of infection via contact from an LA-MRSA-positive outdoor herd

Herds at different addresses could be owned by the same farmer, and transmission among these pig herds was modelled to mimic the contact and potential transmission of LA-MRSA via shared workers or equipment. If a herd was positive for LA-MRSA, transmission was implemented among all herds owned by the same farmer. Therefore, the herd prevalence was calculated for the positive herd and a low-risk transmission rate was used for between-compartment transmission in order to model the spread between the positive herd to the sow (or weaner or finisher) section of the other herds owned by the same farmer (Table 1).

In the original study by Schulz et al.[14], 17 initialisation scenarios were simulated in which LA-MRSA was introduced to pig herds in 2006 and, depending on the scenario, further introductions followed in subsequent years. The predicted herd prevalence was compared to LA-MRSA screening results in 2008 and 2014. We defined a new initialisation scenario for the introduction of LA-MRSA in the first years of the simulation period, aiming at a median herd prevalence between 60% and 70% in 2015. We selected 400 production herds and 10 breeding and multiplier herds at random to be LA-MRSA positive in 2006. A second introduction was modelled in 2009, again by random selection of 400 production herds and 10 breeding and multiplier herds positive for LA-MRSA.

In the following scenarios, the predicted herd prevalence on 31st December 2012 was the basis for comparison among different control strategies.

Control strategies

To simulate different interventions and to evaluate their performance, the initial model developed by Schulz et al.[14] was enhanced. Four control measures were simulated, either separately or in different combinations. All control measures were initiated on 1st January 2007 (initiation date) and continued until the end of the simulation. To investigate the effect of starting date, i.e. the effect of the initial prevalence

of MRSA-positive herds, all scenarios were also run with 1st January 2010 as the initiation date for the simulated control strategy. The results were measured as the prevalence of LA-MRSA-positive herds on 31st December 2012 (for initialisation in 2007) and 31st December 2015 (for initialisation in 2010). This enabled us to compare the effects after a similar time period. If several control measures were combined, all control measures were initiated on the same date. In addition, this allowed us to quantify the effects of control measures under a higher initial LA-MRSA prevalence compared to the initial prevalence in 2007.

The relative reduction was calculated as the proportion R_s for each scenario s, calculated as:

$$R_s = \frac{\overline{Prev_d} - \overline{Prev_s}}{\overline{Prev_d}},$$

with $\overline{Prev_s}$ as the predicted median prevalence of scenario s and $\overline{Prev_d}$ as the predicted median prevalence of the default scenario.

Reduction of high-risk antibiotic use (AB)

The model included information on the prescription of β-lactams or tetracycline at herd level (Schultz et al., 2014). Herds that received prescriptions for these antibiotics were modelled with higher within-herd transmission rates (value high-transmission) compared to herds that did not receive prescriptions (value low-transmission; Table 1). In the scenarios that used this control measure, 50% or 100% of herds that received prescriptions for high-risk antibiotics on the initiation date were randomly chosen. From the initiation date until the end of the simulation period, cessation of high-risk antimicrobial use was simulated by changing the within-herd dynamics of these herds using transmission rates for herds that did not use high-risk antibiotics.

Reduced probability for indirect transmission via humans (ProbIT)

In scenarios using this control measure, we reduced the minimum, maximum and mode value of the default PERT distributions by 50% or 75% in all herds Table 3).

Movement restriction (MR)

To limit the spread of LA-MRSA via pig movements, a potential control option would be to prohibit movements from LA-MRSA-positive to LA-MRSA-negative herds. In this case, the status of the herds must be known. We enhanced the initial model by simulating periodic LA-MRSA screenings within the herds. Testing was simulated by nasal swabs with a sensitivity of 78% and a specificity of 99.9%[18]. We randomly

assigned test results to the simulated herds based on assumed test characteristics of the true status of the herds on the day of an LA-MRSA screening.

For all movements, we then checked the following cases:

1. If the sending herd had a negative test result, pigs were moved according to the movement data.
2. If the sending and receiving herds were simulated to have positive test results, pigs were also moved according to the movement data.
3. If the sending herd had a positive test result, but the receiving herd had a negative test result, we assumed that the pigs were moved to another LA-MRSA-positive herd. For all LA-MRSA-positive herds, we checked for a potential new receiver of the same herd category (dependent on the number of registered sows, weaners and finishers) and randomly selected one, if available. If there were no potential new receivers, we assumed that the pigs were exported and therefore disregarded the movement.

We ran two scenarios to mimic movement restrictions: testing all herds once per year and testing all herds four times per year. We assumed that all herds were tested on the same day.

Voluntary eradication (Erad)

Mimicking an eradication process (i.e., depopulation followed by cleaning and disinfection) also required testing the herds, as only herds testing positive for LA-MRSA would initiate an eradication programme. We assumed that 7.5% of the breeding and multiplier herds and 5% of all other herd types would begin eradication after testing positive for LA-MRSA. These herds were chosen randomly out of all breeding and multiplier (other herds) that tested positive for LA-MRSA. The eradication process lasted between 168 and 378 simulation days, depending on the production type of the herd (Table 4). These values were based on experience of previous eradication programmes performed in Danish pig herds (personal communication, Finn Udesen – SEGES, Danish Agriculture & Food Council). During this time period, no movements (either in or out) were performed. We ignored these movements, assuming that the herd did not send pigs to other herds, except for slaughter or culling. Moreover, we assumed that the herd would have been restocked with LA-MRSA-negative pigs. As soon as the eradication period ended, registered in-coming and out-going movements were modelled as implemented in the original model. We ran this control measure assuming that herds would be screened for LA-MRSA once per year. When voluntary eradication was combined with movement restrictions based on testing all herds four times per year, herds were sampled

four times per year as well as at the start of the eradication process. An upper limit of 25% of all registered breeding and multiplier herds was set. If this limit was exceeded, no additional breeding and multiplier herds initiated eradication. No limit was set for other herd types.

Table 4. Assumed duration of the eradication process, dependent on herd categories based on the registered number of sows and finishers.

Production type	Description	Assumed duration of eradication process
Sow herd	< 5 finishers per sow	266 days
Integrated herd	5-7.7 finishers per sow	378 days
Finisher herd	> 7.5 finishers per sow	168 days

All control strategies were added to the original model individually and combined in all possible combinations.

Simulation modelling and graphical presentation of results were performed in R version 3.2.2 - "Fire Safety"[19]. Like the original model, all simulations were run with 500 iterations to cover any extra variability in the different scenarios.

RESULTS

The default initialisation scenario without control measures led to a predicted median herd prevalence of 47% on 31st December 2012 and 62% on 31st December 2015 (Table 5a). On 1st January 2007, the median prevalence was 4% [90% prediction interval: 3% - 7%]. Control strategies with this initiation date are presented in Table 5a. No reduction in median prevalence was observed when the number of herds using high-risk antibiotics was reduced by 50% (Table 5a, Scenario 1.1: AB (50%)). However, a relative reduction of 38% was observed if all herds reduced the use of high-risk antibiotics (Table 5a, Scenario 1.2: AB (100%)). Furthermore, these scenarios showed larger variation compared to the default scenario. Reducing the probability of effective indirect contact from LA-MRSA-positive herds by 50% or 75% (Scenarios 1.3 and 1.4: ProbIT (75%) and ProbIT (100%)) led to reductions in the median prevalence to 37% and 31%, respectively (Table 5a). Movement restrictions did not lead to a reduction in the predicted median prevalence when it was based on testing herds once per year (Table 5a, Scenario 1.5: MR (1/year)), while a relative reduction of 22% was observed when movement restrictions were based on four

yearly screenings (Table 5a, Scenario 1.6: MR (4/year)). Voluntary eradication (Scenario 1.7: Erad (1/year)) led to a limited reduction in the median prevalence to 43%.

Table 5a. Predicted median prevalence of the default scenario and the four individual control measures 6 years after initiation of the control programme. The scenario acronyms are described in the main text.

Scenario ID	Scenario acronym	Predicted median herd prevalence in % on 31st December 2012 with initialisation of control on 1st January 2007 [90% prediction interval] (relative reduction)	Predicted median herd prevalence in % on 31st December 2015 with initialisation of control on 1st January 2010 [90% prediction interval] (relative reduction)
No control measures			
0	Default	47 [42 - 52]	62 [59 - 65]
Single control measures			
1.1	AB (50%)	47 [30 - 61] (0%)	59 [46 - 70] (6%)
1.2	AB (100%)	29 [13 - 44] (38%)	48 [26 - 60] (24%)
1.3	ProbIT (50%)	37 [31 - 43] (21%)	57 [52 - 60] (9%)
1.4	ProbIT (75%)	31 [26 - 37] (33%)	53 [48 - 57] (15%)
1.5	MR (1/year)	47 [43 - 53] (0%)	63 [60 - 66] (-1%)
1.6	MR (4/year)	37 [32 - 42] (22%)	55 [51 - 58] (12%)
1.7	Erad (1/year)	43 [38 - 49] (8%)	59 [55 - 62] (6%)

In the next step, we combined two control scenarios. We observed an additive effect that was slightly larger when the combination included a reduction in high-risk antibiotic use in all herds (Table 5b, Scenarios 2.6-2.10). The variance increased in all scenarios that included the reduction of high-risk antibiotics. The lowest median herd prevalence (15%) was found when reduced high-risk antibiotic use in 100% of the herds was combined with a 75% reduction in the probability of effective indirect contact from LA-MRSA-positive herds (Table 5b, Scenario 2.7).

The combination of three control measures led to a maximum reduction in the simulated median prevalence to 10% herd prevalence when the reduction of high-risk antibiotics in all herds, movement restrictions based on four tests per year, and voluntary eradication scenarios were combined (Table 5c, Scenario 3.16). In 14 of 20 scenarios, the relative reduction was higher than 50%.

Table 5b. Predicted median prevalence of the default scenario and a combination of two control measures 6 years after the initiation of the control programme. The scenario acronyms are described in the main text.

Scenario ID	Scenario acronym		Predicted median herd prevalence in % on 31st December 2012, initialisation of control on 1st January 2007 [90% prediction interval] (relative reduction)	Predicted median herd prevalence in % on 31st December 2015, initialisation of control on 1st January 2010 [90% prediction interval] (relative reduction)
Combination of two control measures				
2.1	AB (50%)	ProbIT (50%)	36 [22 - 49] (24%)	52 [39 - 64] (16%)
2.2		ProbIT (75%)	30 [17 - 42] (37%)	49 [36 - 60] (22%)
2.3		MR (1/year)	47 [30 - 61] (0%)	59 [48 - 70] (5%)
2.4		MR (4/year)	35 [22 - 50] (25%)	51 [38 - 63] (17%)
2.5		Erad (1/year)	42 [26 - 56] (10%)	55 [41 - 67] (12%)
2.6	AB (100%)	ProbIT (50%)	22 [11 - 35] (54%)	41 [23 - 55] (34%)
2.7		ProbIT (75%)	15 [7 - 26] (68%)	38 [22 - 50] (40%)
2.8		MR (1/year)	30 [15 - 46] (36%)	48 [28 - 63] (22%)
2.9		MR (4/year)	21 [9 - 33] (56%)	38 [20 - 50] (39%)
2.10		Erad (1/year)	26 [11 - 40] (45%)	41 [24 - 57] (34%)
2.11	ProbIT (50%)	MR (1/year)	38 [32 - 43] (19%)	58 [53 - 61] (8%)
2.12		MR (4/year)	28 [23 - 33] (41%)	49 [44 - 53] (22%)
2.13		Erad (1/year)	33 [28 - 39] (29%)	52 [48 - 56] (16%)
2.14	ProbIT (75%)	MR (1/year)	32 [27 - 39] (31%)	54 [49 - 58] (14%)
2.15		MR (4/year)	23 [19 - 29] (50%)	45 [40 - 49] (28%)
2.16		Erad (1/year)	28 [23 - 34] (41%)	48 [42 - 52] (24%)
2.17	MR (1/year)	Erad (1/year)	29 [25 - 34] (37%)	46 [42 - 49] (26%)
2.18	MR (4/year)	Erad (4/year)	21 [18 - 25] (55%)	36 [32 - 40] (42%)

Table 5c. Predicted median prevalence of the default scenario and a combination of three control measures 6 years after the initiation of the control programme. The scenario acronyms are described in the main text.

Scenario ID	Scenario acronym			Predicted median herd prevalence in % on 31st December 2012 [90% prediction interval] (relative reduction)	Predicted median herd prevalence in % on 31st December 2015 [90% prediction interval] (relative reduction)
Combination of three control measures					
3.1	AB (50%)	ProbIT (50%)	MR (1/year)	36 [22 - 49] (23%)	53 [40 - 65] (14%)
3.2			MR (4/year)	26 [16 - 38] (44%)	44 [32 - 56] (29%)
3.3			Erad (1/year)	32 [19 - 46] (31%)	48 [34 - 61] (23%)
3.4		ProbIT (75%)	MR (1/year)	32 [20 - 43] (33%)	50 [38 - 61] (20%)
3.5			MR (4/year)	21 [13 - 33] (55%)	40 [28 - 50] (36%)
3.6			Erad (1/year)	26 [15 - 38] (44%)	44 [31 - 55] (30%)
3.7		MR (1/year)	Erad (1/year)	29 [18 - 41] (39%)	42 [32 - 53] (32%)
3.8		MR (4/year)	Erad (4/year)	20 [11 - 31] (57%)	32 [21 - 42] (48%)
3.9	AB (100%)	ProbIT (50%)	MR (1/year)	22 [10 - 35] (53%)	42 [25 - 56] (32%)
3.10			MR (4/year)	15 [7 - 25] (68%)	33 [14 - 44] (48%)
3.11			Erad (1/year)	38 [32 - 43] (61%)	35 [20 - 49] (43%)
3.12		ProbIT (75%)	MR (1/year)	19 [7 - 30] (59%)	38 [22 - 49] (39%)
3.13			MR (4/year)	13 [6 - 22] (72%)	29 [16 - 39] (53%)
3.14			Erad (1/year)	15 [5 - 26] (68%)	31 [16 - 43] (50%)
3.15		MR (1/year)	Erad (1/year)	16 [8 - 28] (65%)	30 [15 - 41] (52%)
3.16		MR (4/year)	Erad (4/year)	10 [4 - 18] (79%)	21 [10 - 31] (67%)
3.17	ProbIT (50%)	MR (1/year)	Erad (1/year)	21 [17 - 25] (55%)	39 [34 - 42] (38%)
3.18		MR (4/year)	Erad (4/year)	15 [12 - 17] (69%)	29 [25 - 33] (53%)
3.19	ProbIT (75%)	MR (1/year)	Erad (1/year)	17 [14 - 21] (64%)	34 [29 - 39] (46%)
3.20		MR (4/year)	Erad (4/year)	12 [9 - 14] (75%)	25 [21 - 29] (60%)

Combining all four control measures showed the highest potential to reduce the simulated prevalence compared to the default scenario (Table 5d). The smallest median prevalence was estimated at 6% (90% prediction interval: 2% - 10%) and was observed with the combination of antibiotic reduction in all herds, a 75% reduction in the probability of effective indirect transmission via humans, movement restrictions based on testing all herds four times per year and voluntary eradication (Table 5d, Scenario 4.8). LA-MRSA was not cleared from all simulated herds following the set-up of control measures in any of the simulation scenarios.

Simulating control measure initiation on 1st January 2010 led to comparable tendencies in the effects of single interventions and combinations of the tested control options. The median prevalence on the initiation date was 21% [90% prediction interval: 18% - 34%]. In all scenarios, the predicted herd prevalence was higher when control was initiated in 2010, compared to the scenarios with an initiation date of 1st January 2007 (Tables 5a-d). The smallest median herd prevalence 6 years after initiation in 2010 was 13% (90% prediction interval: 5% - 19%), compared to 6% (90% prediction interval: 2% - 10%) for controls initiated in 2007 in the scenario combining all four control measures (Scenario 4.8). Initiating control measures in 2007 generally led to higher relative reduction rates than the same control strategy started in 2010 (Tables 5a-d). However, reduction rates were still fairly high when the combination of four control measures was initiated in 2010.

Table 5d. Predicted median prevalence of the default scenario and the combination of four control measures 6 years after the initiation of the control programme. The scenario acronyms are described in the main text.

Scenario ID	Scenario acronym				Predicted median herd prevalence in % on 31st December 2012 [90% prediction interval] (relative reduction)	Predicted median herd prevalence in % on 31st December 2015 [90% prediction interval] (relative reduction)
Combination of four control measures						
4.1	AB (50%)	ProbIT (50%)	MR (1/year)	Erad (1/year)	20 [12 - 30] (56%)	34 [24 - 44] (45%)
4.2			MR (4/year)	Erad (4/year)	13 [8 - 22] (72%)	25 [16 - 34] (61%)
4.3	AB (50%)	ProbIT (75%)	MR (1/year)	Erad (1/year)	16 [9 - 24] (65%)	30 [21 - 38] (53%)
4.4			MR (4/year)	Erad (4/year)	11 [6 - 16] (77%)	21 [13 - 30] (66%)
4.5	AB (100%)	ProbIT (50%)	MR (1/year)	Erad (1/year)	11 [5 - 19] (77%)	23 [14 - 33] (62%)
4.6			MR (4/year)	Erad (4/year)	7 [3 - 13] (86%)	16 [6 - 24] (74%)
4.7	AB (100%)	ProbIT (75%)	MR (1/year)	Erad (1/year)	9 [5 - 15] (80%)	21 [11 - 29] (66%)
4.8			MR (4/year)	Erad (4/year)	6 [2 -10] (86%)	13 [5 - 19] (79%)

Figure 2 shows the development of the predicted LA-MRSA herd prevalence for the default scenario (Scenario: 0), and for the scenario with the highest reduction in LA-MRSA herd prevalence (Scenario: 4.8) for both of the control strategy initiation dates.

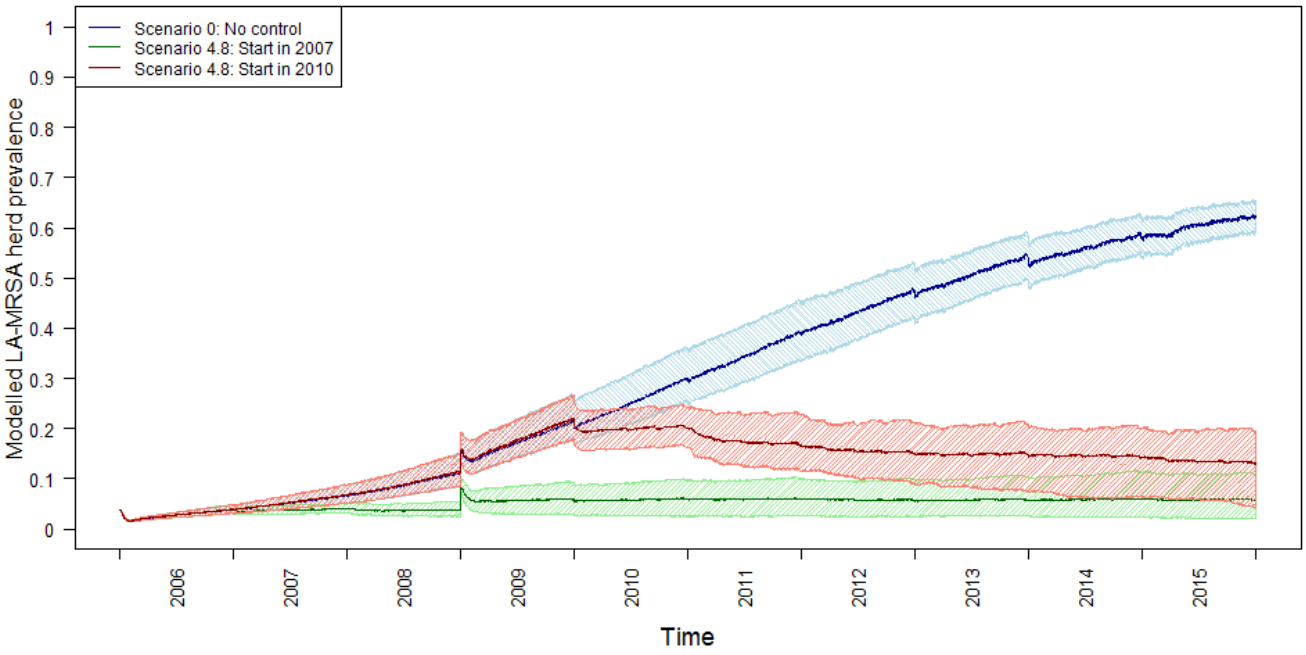

Figure 2. Predicted LA-MRSA herd prevalence over the whole study period from 1st January 2006 to 31st December 2015 for the following three scenarios: (1) Default (blue), (2) Scenario 4.8 (AB (100%) + ProbIT (75%) + MR (4/year) + Erad (4/year)) for control measures starting on 1st January 2007 (green), and (3) Scenario 4.8 for control measures starting on 1st January 2010 (red). Dark lines represent the predicted median herd prevalence, the light dashed areas represent the 90% prediction interval. The scenario acronyms are described in the main text.

DISCUSSION

LA-MRSA spread among Danish pig herds was modelled using four potential control options. The results showed that initiating intensive and combined control measures in 2007 would have led to a slower increase in the LA-MRSA herd prevalence (Tables 5a-d). In particular, the combination of all four implemented control measures showed the potential to limit the spread among pig herds. Nevertheless, LA-MRSA was not cleared from all herds during the study period for any of the tested scenarios. Initiating control measures in 2010 also showed a reduction in the predicted herd prevalence of LA-MRSA. However, the relative reductions were smaller when compared to simulating the start of control in 2007 (Table 6).

Reducing the use of high-risk antibiotics such as β-lactams and tetracycline has been shown to reduce transmission rates for the within-herd spread of LA-MRSA[15]. We found that reducing the proportion of herds using high-risk antibiotics had a limiting effect on the between-herd spread of LA-MRSA. This might be related to the lower within-herd prevalence and thus to a lower risk of LA-MRSA transmission among herds. Our results correspond to the findings of Sørensen et al.[20], who used a mechanistic simulation model to show that reducing antimicrobial consumption reduced the prevalence of LA-MRSA within a farrow-to-finisher herd, but that bacteria was not eradicated. Reducing the within-herd prevalence would reduce the probability of infection following contact with a susceptible herd, leading to a reduction in the between-herd prevalence. However, as the transmission rate is not zero after a reduction in the use of high-risk antibiotics[15], the pathogen can still spread and hence eradication did not occur.

All scenarios that included a reduction of high-risk antibiotics led to increased variance in the predicted prevalence on 31st December 2015. Low transmission rates might have led to a higher number of herds in which LA-MRSA faded out after introduction. More precisely, LA-MRSA might have been cleared from pigs after the introduction of control measures and before transmission within or between the compartments occurred. This effect might therefore have reduced the spread of LA-MRSA among pig herds and resulted in lower predicted herd prevalences. On the other hand, if LA-MRSA was established in a pig herd, the within-herd prevalence reached a similar level to that of herds using high-risk antibiotics[14], meaning that the spread from these herds to other herds was not affected. This might explain the larger variation in herd prevalence at the end of the study period compared to the default scenario.

Reducing the probability of indirect LA-MRSA transmission among pig herds could be interpreted as biosecurity measures for humans visiting more than one herd on the same day. This control measure also showed a limiting effect on the between-herd spread (Table 2). Humans visiting a pig herd could carry LA-MRSA for a period of a few hours up to 2 days[21]. Regulations to ensure that farm visitors (veterinarians, advisors, technicians, guests) wear masks might help to lower the risk of transmission of LA-MRSA to another farm[21].

Although pig movements might play a role in the transmission of LA-MRSA among pig herds[10,11,14,22], movement restrictions only seemed to lead to a marginal reduction in herd prevalence. Additionally, it would be necessary to test all herds to ensure that this intervention was effective. This would require logistical and financial resources that might not be reasonable in relation to the effects predicted by the

model. In addition, there is no perfect method for testing herds for LA-MRSA; in the current model, a sensitivity of 78% and a specificity of 100% were used. Despite the high specificity of the available tests[18], the sensitivity is relatively low, leading to many false negative results. False-negative herds may jeopardise the movement restrictions and might be responsible for the low effectiveness of this strategy.

Eradication (i.e., depopulation followed by cleaning and disinfection) of LA-MRSA in herds that test positive for LA-MRSA could be an option to reduce the herd prevalence. We assumed that only small proportions of herds that tested positive for LA-MRSA would initiate an eradication programme. Depopulation and re-stocking large proportions of pig herds might lead to ethical and economic issues. In Norway, where the prevalence of LA-MRSA was low, eradication at herd level helped to limit the spread of LA-MRSA on a national level[23]. Voluntary eradication only marginally reduced the prevalence. However, eradication without the combination of movement restrictions poses the risk of re-introduction, especially when the herd prevalence is high, which might explain the low effectiveness of this control measure. In contrast, voluntary eradication combined with movement restrictions based on four tests per year led to one of the largest reductions in the predicted herd prevalence. This example highlights how intensive control measures might have had reduced the spread of LA-MRSA in Denmark. However, increasing the proportion of herds to be eradicated might be unrealistic as it would affect Danish pig production and lead to substantial economic losses[24].

The effects of eradication on the herd prevalence are highly dependent on the proportion of herds that initiate an eradication process. Cost-benefit analyses must be included in the decision process when setting up control programmes that involve eradication efforts. In addition, the risk of re-introduction must be taken into account, for example by combining eradication with movement restrictions to minimise this risk. The role of environmental contamination in the spread of LA-MRSA has not yet been conclusively determined and therefore might influence the effectiveness of control programmes.

Herds initiating the voluntary eradication process were chosen randomly. It is therefore possible that herds registering no or only a few out-going contacts might have been selected. Prioritising herds with a high number of out-going movements or with a large out-going contact chain might increase the effects of the eradication process, as clearing these herds would prevent more herds from receiving LA-MRSA via animal movements.

For simplicity, we assumed that all herds were tested on the same day when the herd LA-MRSA status was established. In reality, herds would be tested within a certain time period (of 3 months or 1 year). This could influence the effect of movement restrictions and voluntary eradication as varying the time points at which LA-MRSA-positive herds are identified might result in control measures starting later, and transmission would still be possible until initiation of the eradication process.

We assumed that the control measures initialised in 2007 did not influence the second wave of LA-MRSA introduction in randomly chosen herds in 2009. Despite phylogenetic analysis confirming several introductions of LA-MRSA to Denmark[25], the route of these introductions is still unknown. The effect of control measures on new introductions could therefore not be estimated. For example, LA-MRSA-positive workers could have introduced LA-MRSA in new herds, as described in Norway[23]. This route of introduction was not necessarily covered by the implemented control measures, depending on how a worker would carry the bacteria into a herd. As a result, introduction was still assumed to be possible, even under the implemented control measures.

We compared the effects of control measures starting on 1st January 2007 and 1st January 2010, and found that the early initialisation of control measures led to a larger reduction in the predicted herd prevalence on 31st December 2012 compared to 31st December 2015 (Tables 5a-d). This may not be surprising, as disease control in 2007 started at a lower herd prevalence compared to initialisation in 2010 (Figure 2). However, starting the control measures in 2010 still led to reasonably high relative reduction rates (Tables 5a-d), despite an initial prevalence of 21%. The prevalence in 2016 was substantially higher at 88%[3]. Therefore, the effectiveness of these strategies from a high initial prevalence should be investigated to understand which measures or combinations could be useful in a situation with a very high prevalence, such as the current Danish situation. However, this would require a different approach to modelling animal movements, as data on registered animal movements are only available retrospectively. We therefore emphasise the importance of a region/country with a new introduction of LA-MRSA controlling/eradicating it immediately in order to prevent an endemic situation with a high prevalence.

Combinations of control measures reduced the spread of LA-MRSA, especially when all four strategies were combined. Using an extreme scenario including limiting the use of high-risk antibiotics, reducing the risk of spread via indirect contact by 75%, implementing movement restriction and culling a percentage of positive herds led to a prevalence reduction to only 6% with initiation in 2007 or 13% in 2010. This clearly

shows that control of LA-MRSA can be achieved without culling all infected herds. However, it requires extreme measures, willingness from the industry and rigour in implementing these measures, otherwise high relative reduction rates might not be reached. In addition, it is important to initiate control measures as early as possible, as the effects are higher if the herd prevalence is still low.

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AUTHOR CONTRIBUTIONS

N.T., T.H. and A.B. devised the project; J.S. performed the simulations and calculations; A.B. and T.H. supervised the project; all authors interpreted the results and produced the final manuscript.

ADDITIONAL INFORMATION

Competing financial interests: the authors declare no competing financial or non-financial interests.

4. RESULTS AND DISCUSSION

4.1. NETWORK ANALYSIS (MANUSCRIPT I)

A variety of static and dynamic network analysis tools are available for investigating animal trade networks[69]. Manuscript I (Chapter 3.1) focused on a general description of the investigated pig movement network, loyalty patterns and the size of out-going contact chains of the different holding types registered in the CHR during the study period from 1st January 2006 to 31st December 2015.

A decrease in the number but increase in the size of holdings was observed during the considered time period. The frequency of movements decreased over time, which is likely to be related to the decreasing number of active holdings.

The frequency of contact with other holdings was influenced by the registered holding type. In addition, the holding type of the receiving herd was influenced by the holding type of the sender. These influences reflect the pyramidal structure of the Danish pig industry.

Clear loyalty patterns for different holding types were observed, yet there was also variability among holdings of the same type. Weaner herds showed the highest level of in-loyalty. Boar stations and production herds were characterised by a high level of out-loyalty.

The giant strongly connected components (GSCCs) included less than 1% of active holdings, mainly production herds. This indicated fewer horizontal connections in the pig production pyramid.

All production sites showed low levels of incoming contact chains. A high level of out-going contact chain was observed for breeding and multiplier herds, reflecting the fact that these herds form the top of the production pyramid and thus pose a higher potential risk of spreading a disease throughout the entire pig trade network.

Holdings combining a low/intermediate out-loyalty and a high level of out-going contact chain might have a higher risk of spreading disease. This combination was shown in breeding and multiplier herds, and once a pathogen is introduced, the risk of spreading the pathogen along the production line is expected to be high. However, breeding and multiplier herds have high levels of biosecurity and the introduction of pathogens might therefore be lower compared to other herd types.

4.2. Simulation Modelling (Manuscript II)

4.2.1. Additional results

To investigate the impact of each of the modelled transmission routes (animal movements, indirect contact via humans, abattoir movements and shared ownership of herds) on the spread of LA-MRSA, we calculated the proportion of each transmission route. Figure 1 shows these proportions for the default scenario presented in Manuscript II (Chapter 3.2). Animal movements were responsible for around 74% of the transmissions (newly affected herds) in our model. Indirect contact, which was shown to be essential in mimicking the rapid increase of LA-MRSA-positive herds in Denmark, was responsible for around 20% of transmissions (newly affected herds). Abattoir movements (around 5%) and transmission between herds with the same owner (around 1%) are predicted to play a minor role in the spread of LA-MRSA.

Halving or doubling the frequency of indirect contact or the probability of an effective transmission from an LA-MRSA-positive indoor or outdoor herd led to comparable results in the proportions of the four modelled transmission pathways. Halving (doubling) the parameters led to an increase (decrease) in the median proportion of transmissions via pig movements to around 81% (65%, Figure 2). In both cases, pig movements constitute the largest proportion of LA-MRSA transmissions in the model.

Halving (doubling) the probability of infection via abattoir movements led to an increase (decrease) of 2% in the median proportion of transmissions via animal movements (Figure 3).

Pig movements were shown to be the transmission route with the highest proportion of transmissions among pig herds, irrespective of the scenario considered (Figures 1-3).

4.2.2. Discussion

Livestock movements have been discussed as a driving factor in the transmission of LA-MRSA among pig herds, and a simulation model was therefore developed to mimic the LA-MRSA situation in Denmark between January 2006 and December 2015. Scenarios were modelled to simulate the spread via pig movements alone, as well as combined with indirect transmissions via humans, abattoir movements and between herds with the same owner. Several initialisation scenarios were run, as the exact time of the introduction to Denmark and therefore the true status in 2006 was unknown.

In the simulation model, breeding and multiplier herds played a key role in the initialisation process. LA-MRSA was initialised in only one to five breeding and multiplier herds in 2006, but more than 100 production herds were necessary to reach a similar herd prevalence at the end of the study period. Additionally, LA-MRSA was also introduced in later years in several scenarios to reflect unknown introductions. Sieber et al.[84] described three main clones of LA-MRSA circulating in Denmark, which were introduced at different time points. We aimed to demonstrate a new introduction in 2009 by choosing a scenario with initialisation in 2006 and 2009 as a default. These new introductions represented introductions from unknown sources, such as from newly employed workers carrying LA-MRSA and transmitting the pathogen to pigs[53].

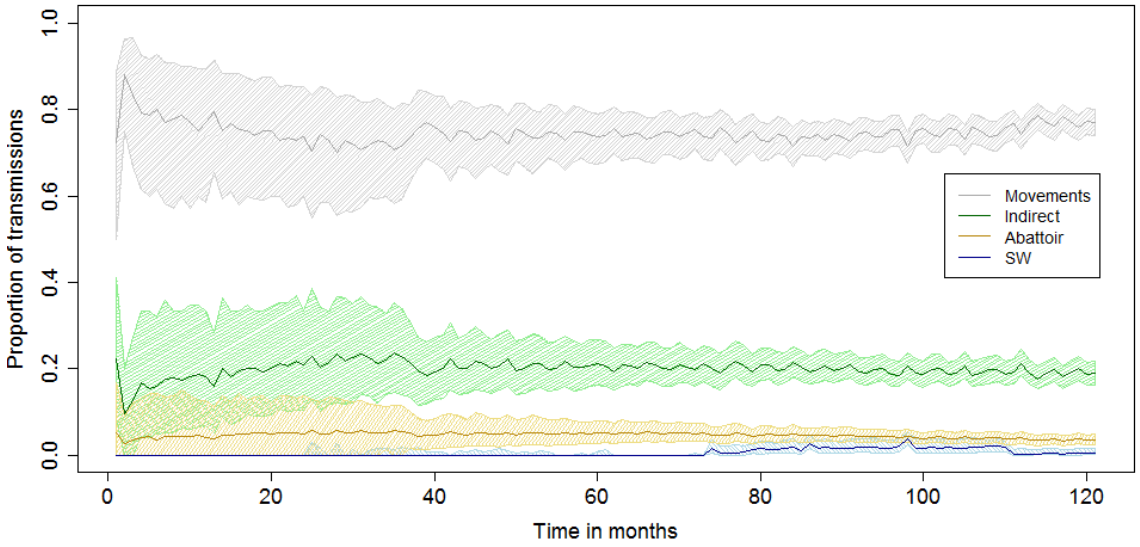

FIGURE 1. Proportion of modelled transmissions (newly affected herds) of LA-MRSA: (1) via pig movements (Movements - grey), (2) via indirect transmissions via humans from indoor and outdoor herds (Indirect - green), (3) related to abattoir movements (Abattoir - orange), and (4) between herds with the same owner via shared workers or equipment (SW - blue). The lines represent the median of 500 simulation repetitions; the hatched area represents the 90% prediction interval.

The model results showed that pig movements alone were not sufficient to mimic the rapid increase of LA-MRSA-positive herds in Denmark (Manuscript II). However, even extensive initialisation in 2006, as well as in 2006 and 2009 was not sufficient to mimic the situation in Danish pig herds. Adding indirect contact showed that the model was able to mimic the observed trend in LA-MRSA prevalence in Denmark. However, in these scenarios, the predicted herd prevalence in 2008 was higher than the LA-MRSA screening results. Scenarios that involved doubling the frequency or probability of effective indirect transmission via humans showed a similar trend to that observed in Denmark during the study period.

Results of the simulation models are dependent on the model parameters used. While on the one hand there is literature available regarding parameters like transmission rates in pig herds, there is a lack of knowledge relating to other parameters essential to the model development process. An example is the lack of details on the effectiveness of transmission via indirect contact. An extensive sensitivity analysis was performed to investigate the influence of uncertain parameters. This sensitivity analysis highlighted the influence of the within-herd prevalence and the frequency and effectiveness of indirect transmission via humans as key parameters for the spread of LA-MRSA among herds. This highlights the need for more research to improve our understanding of the dynamics of LA-MRSA within pig herds and the role of humans in its spread, in order to develop an effective control strategy for LA-MRSA at a national level.

Assuming homogeneous mixing in small herds did not influence the predicted herd prevalence. It is likely that small herds have the same characteristics as hobby herds in terms of trade contacts. In general, they have few trade contacts and might therefore play a minor role in the spread of LA-MRSA.

Varying the parameters related to environmental re-introduction of LA-MRSA to negative herds that were previously LA-MRSA positive also did not influence the predicted herd prevalence. However, we have little knowledge about environmental transmission, so the effect could be influenced by the method of implementation. More knowledge about the impact of the environment is therefore needed to draw accurate conclusions about its effects.

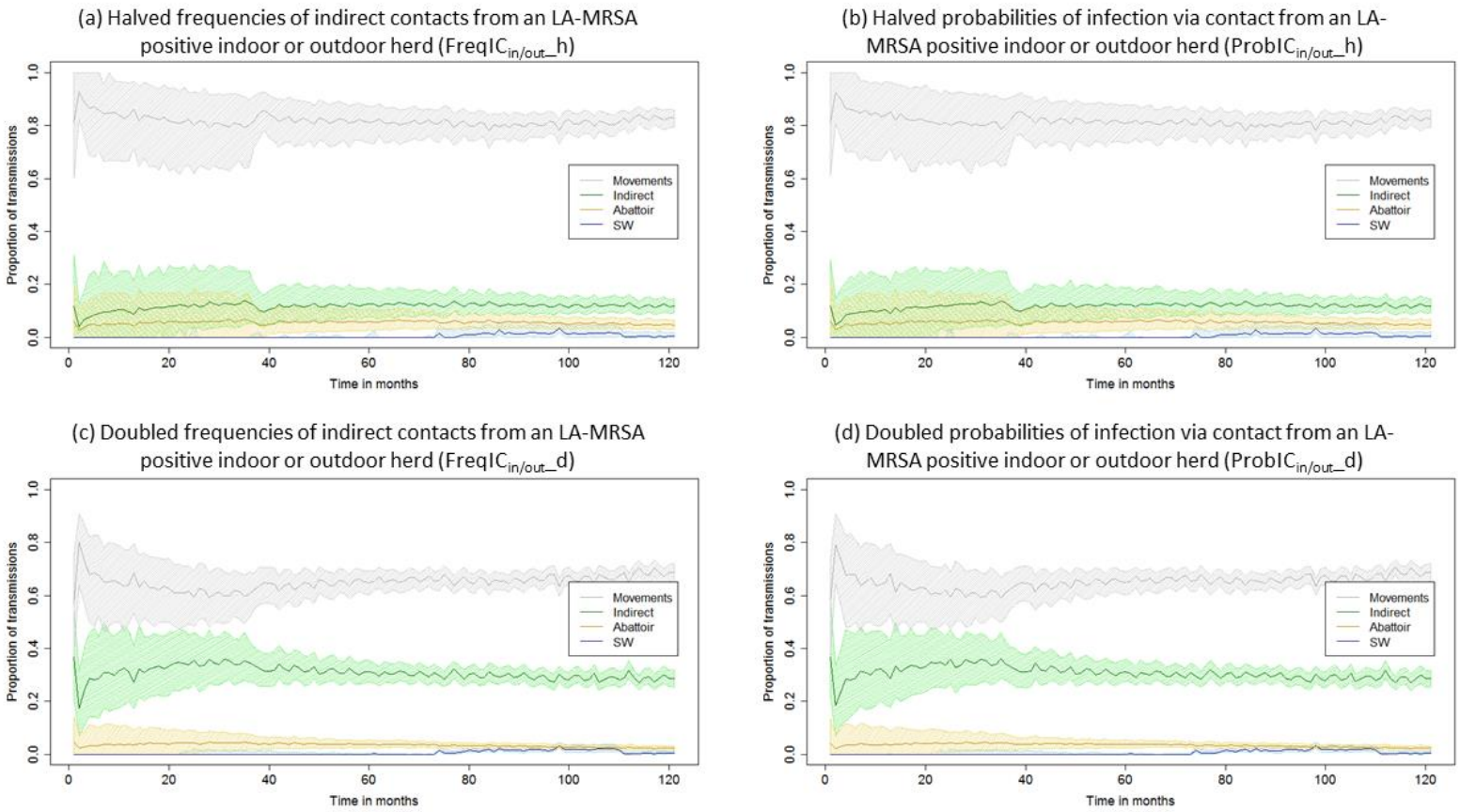

FIGURE 2. Proportions of modelled transmissions (newly affected herds) of LA-MRSA in four scenarios run in the framework of the sensitivity analysis: (a+c) halved and doubled frequencies of indirect contact from an LA-MRSA-positive indoor or outdoor herd and (b+d) halved and doubled probabilities of infection via contact from an LA-MRSA-positive indoor or outdoor herd. The lines represent the median of 500 simulation repetitions; the hatched area represents the 90% prediction interval.

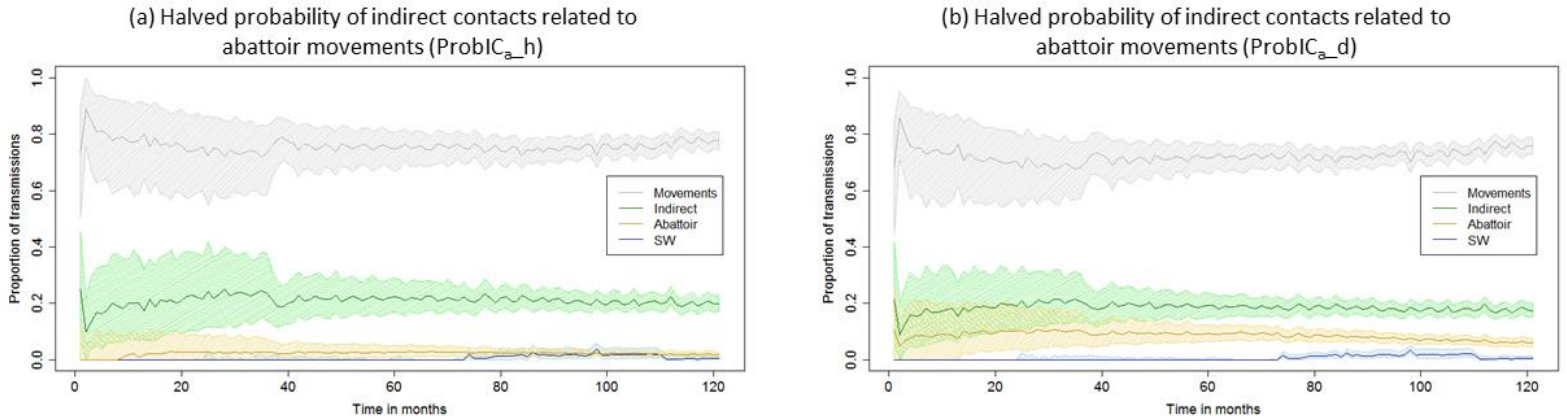

FIGURE 3. Proportion of modelled transmissions (newly affected herds) of LA-MRSA: (1) via pig movements (Movements - grey), (2) via indirect transmission via humans from indoor and outdoor herds (Indirect - green), (3) related to abattoir movements (Abattoir - orange) and (4) between herds with the same owner via shared workers or equipment (SW - blue). The lines represent the median of 500 simulation repetitions; the hatched area represents the 90% prediction interval.

4.3. Modelling control measures (Manuscript III)

4.3.1. Additional results

4.3.1.1. Predicted herd prevalence on 31st December 2015

All scenarios presented in Manuscript III were run until 31st December 2015. If control measures were implemented individually, an increase in the predicted median herd prevalence could be observed from 2012 to 2015 in all scenarios (Table 1a). The relative reduction compared to the default scenario without control measures decreased, except for the scenario with reduced antibiotic usage in 50% of the herds (Scenario 1.1).

Table 1a. Predicted median prevalence on 31st December 2012 and 31st December 2015 for the default scenario presented in Manuscript III. All control measures were implemented individually.

Scenario ID	Scenario acronym	Predicted median herd prevalence in % on 31st December 2012* [90% prediction interval] (relative reduction)	Predicted median herd prevalence in % on 31st December 2015 [90% prediction interval] (relative reduction)
No control measures			
0	Default	47 [42 - 52]	62 [59 - 65]
Single control measures			
1.1	AB (50%)	47 [30 - 61] (0%)	60 [46 - 70] (4%)
1.2	AB (100%)	29 [13 - 44] (38%)	42 [16 - 59] (32%)
1.3	ProbIT (50%)	37 [31 - 43] (21%)	55 [50 - 59] (12%)
1.4	ProbIT (75%)	31 [26 - 37] (33%)	48 [42 - 53] (23%)
1.5	MR (1/year)	47 [43 - 53] (0%)	62 [59 - 65] (0%)
1.6	MR (4/year)	37 [32 - 42] (22%)	53 [50 - 57] (14%)
1.7	Erad (1/year)	43 [38 - 49] (8%)	58 [54 - 61] (7%)

* Results presented in Manuscript III

Combining two control measures did not stop the increase of the herd prevalence between 2012 and 2015 (Figure 4). The relative reduction observed in 2015 was lower compared to the relative reduction observed in 2012 in all scenarios except Scenarios 2.3 and 2.5, in which there was a slight increase (Table 1b). When combining three control measures, we again found that the predicted herd prevalence increased at least slightly between 2012 and 2015 in all scenarios (Table 1c).

When combining four control measures, the predicted herd prevalence remained constant in Scenario 4.8 (Table 1d, Figure 5), while the relative reduction increased in Scenarios 4.4 - 4.8 (Table 1d).

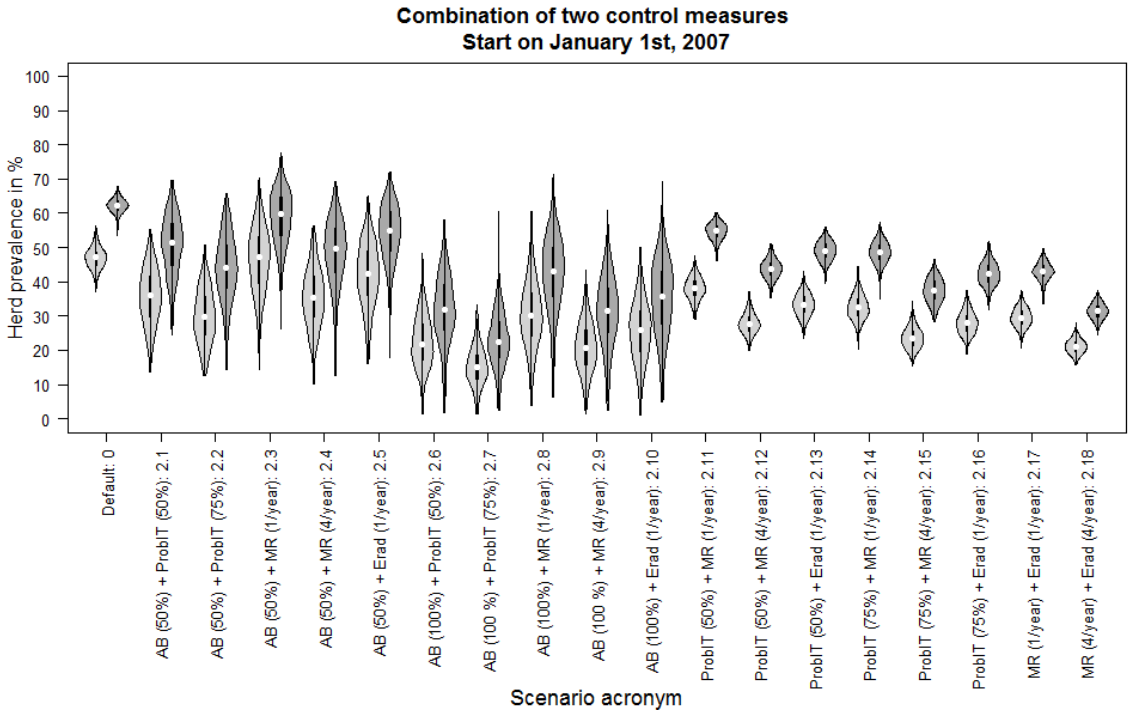

FIGURE 4. Predicted LA-MRSA herd prevalence on 31st December 2012 (light grey) and 31st December 2015 (dark grey) for two combined control measures (scenario acronyms followed by scenario identification number). Control measures were set to start on 1st January 2007. The scenario acronyms are explained in Manuscript III (Chapter 3.2).

TABLE 1B. Predicted median prevalence on 31st December 2012 and 31st December 2015 for the default scenario presented in Manuscript III. All combinations of two control measures were implemented.

Scenario ID	Scenario acronym		Predicted median herd prevalence in % on 31st December 2012* [90% prediction interval] (relative reduction)	Predicted median herd prevalence in % on 31st December 2015 [90% prediction interval] (relative reduction)
No control measures				
0	Default		47 [42 - 52]	62 [59 - 65]
Combination of two control measures				
2.1		ProbIT (50%)	36 [22 - 49] (24%)	52 [36 - 64] (17%)
2.2		ProbIt (75%)	30 [17 - 42] (37%)	44 [29 - 59] (29%)
2.3	AB (50%)	MR (1/year)	47 [30 - 61] (0%)	60 [45 - 70] (4%)
2.4		MR (4/year)	35 [22 - 50] (25%)	50 [33 - 62] (20%)
2.5		Erad (1/year)	42 [26 - 56] (10%)	55 [39 - 66] (12%)
2.6		ProbIT (50%)	22 [11 - 35] (54%)	32 [14 - 47] (49%)
2.7		ProbIT (75%)	15 [7 - 26] (68%)	22 [10 - 36] (64%)
2.8	AB (100%)	MR (1/year)	30 [15 - 46] (36%)	43 [20 - 58] (31%)
2.9		MR (4/year)	21 [9 - 33] (56%)	32 [13 - 47] (49%)
2.10		Erad (1/year)	26 [11 - 40] (45%)	36 [16 - 51] (42%)
2.11		MR (1/year)	38 [32 - 43] (19%)	55 [50 - 58] (12%)
2.12	ProbIT (50%)	MR (4/year)	28 [23 - 33] (41%)	44 [39 - 48] (30%)
2.13		Erad (1/year)	33 [28 - 39] (29%)	49 [44 - 53] (21%)
2.14		MR (1/year)	32 [27 - 39] (31%)	49 [43 - 54] (22%)
2.15	ProbIT (75%)	MR (4/year)	23 [19 - 29] (50%)	38 [32 - 43] (40%)
2.16		Erad (1/year)	28 [23 - 34] (41%)	42 [37 - 48] (32%)
2.17	MR (1/year)	Erad (1/year)	29 [25 - 34] (37%)	43 [39 - 47] (31%)
2.18	MR (4/year)	Erad (4/year)	21 [18 - 25] (55%)	31 [28 - 35] (50%)

* Results presented in Manuscript III

TABLE 1C. Predicted median prevalence on 31st December 2012 and 31st December 2015 for the default scenario presented in Manuscript III. All combinations of three control measures were implemented.

Scenario ID	Scenario acronym			Predicted median herd prevalence in % on 31st December 2012* [90% prediction interval] (relative reduction)	Predicted median herd prevalence in % on 31st December 2015 [90% prediction interval] (relative reduction)
No control measures					
0	Default			47 [42 - 52]	62 [59 - 65]
Combination of three control measures					
3.1	AB (50%)	ProbIT (50%)	MR (1/year)	36 [22 - 49] (23%)	51 [34 - 63] (17%)
3.2			MR (4/year)	26 [16 - 38] (44%)	41 [26 - 53] (35%)
3.3			Erad (1/year)	32 [19 - 46] (31%)	46 [29 - 60] (27%)
3.4		ProbIT (75%)	MR (1/year)	32 [20 - 43] (33%)	46 [30 - 59] (26%)
3.5			MR (4/year)	21 [13 - 33] (55%)	33 [20 - 48] (47%)
3.6			Erad (1/year)	26 [15 - 38] (44%)	39 [24 - 52] (38%)
3.7		MR (1/year)	Erad (1/year)	29 [18 - 41] (39%)	40 [26 - 52] (36%)
3.8		MR (4/year)	Erad (4/year)	20 [11 - 31] (57%)	28 [16 - 41] (55%)
3.9	AB (100%)	ProbIT (50%)	MR (1/year)	22 [10 - 35] (53%)	32 [13 - 47] (48%)
3.10			MR (4/year)	15 [7 - 25] (68%)	22 [10 - 36] (64%)
3.11			Erad (1/year)	38 [32 - 43] (61%)	26 [11 - 42] (58%)
3.12		ProbIT (75%)	MR (1/year)	19 [7-30] (59%)	28 [12 - 42] (56%)
3.13			MR (4/year)	13 [6 - 22] (72%)	19 [7 - 29] (70%)
3.14			Erad (1/year)	15 [5 - 26] (68%)	21 [8 - 34] (67%)
3.15		MR (1/year)	Erad (1/year)	16 [8 - 28] (65%)	23 [9 - 35] (63%)
3.16		MR (4/year)	Erad (4/year)	10 [4 - 18] (79%)	12 [4 - 22] (80%)
3.17	ProbIT (50%)	MR (1/year)	Erad (1/year)	21 [17 - 25] (55%)	32 [28 - 36] (48%)
3.18		MR (4/year)	Erad (4/year)	15 [12 - 17] (69%)	21 [17 - 25] (66%)
3.19	ProbIT (75%)	MR (1/year)	Erad (1/year)	17 [14 - 21] (64%)	25 [21 - 30] (59%)
3.20		MR (4/year)	Erad (4/year)	12 [9 - 14] (75%)	16 [13 - 20] (74%)

* Results presented in Manuscript III

TABLE 1D. Predicted median prevalence on 31st December 2012 and 31st December 2015 for the default scenario presented in Manuscript III. All combinations of four control measures were implemented.

Scenario ID	Scenario acronym				Predicted median herd prevalence in % on 31st December 2012* [90% prediction interval] (relative reduction)	Predicted median herd prevalence in % on 31st December 2015 [90% prediction interval] (relative reduction)
No control measures						
0	Default				47 [42 - 52]	62 [59 - 65]
Combination of four control measures						
4.1	AB (50%)	ProbIT (50%)	MR (1/year)	Erad (1/year)	20 [12 - 30] (56%)	30 [18 - 42] (52%)
4.2			MR (4/year)	Erad (4/year)	13 [8 - 22] (72%)	18 [9 - 29] (71%)
4.3	AB (50%)	ProbIT (75%)	MR (1/year)	Erad (1/year)	16 [9 - 24] (65%)	23 [12 - 35] (62%)
4.4			MR (4/year)	Erad (4/year)	11 [6 - 16] (77%)	13 [7 - 22] (79%)
4.5	AB (100%)	ProbIT (50%)	MR (1/year)	Erad (1/year)	11 [5 - 19] (77%)	14 [5 - 24] (77%)
4.6			MR (4/year)	Erad (4/year)	7 [3 - 13] (86%)	8 [3 - 14] (88%)
4.7	AB (100%)	ProbIT (75%)	MR (1/year)	Erad (1/year)	9 [5 - 15] (80%)	12 [6 - 19] (81%)
4.8			MR (4/year)	Erad (4/year)	6 [2 -10] (86%)	6 [2 - 11] (91%)

* Results presented in Manuscript III

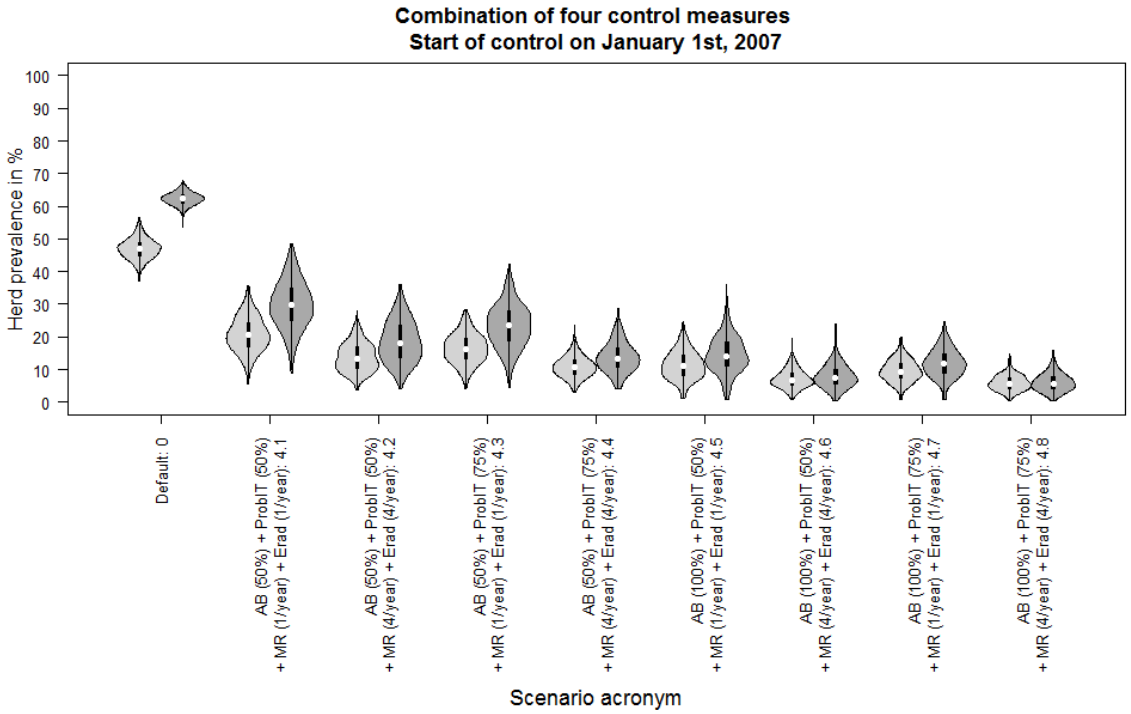

FIGURE 5. Predicted LA-MRSA herd prevalence on 31st December 2012 (light grey) and 31st December 2015 (dark grey) for the combination of four control measures (scenario acronyms followed by scenario identification number). Control measures were set to start on 1st January 2007. The scenario acronyms are explained in Manuscript III (Chapter 3.3).

4.3.1.2. ERADICATION OF HUBS

Running all scenarios including the eradication process based on removing hubs led to a clear initial reduction in the LA-MRSA herd prevalence on 31st December 2012 compared to the scenarios run with random voluntary eradication (Table 2). The relative reduction increased for all scenarios. An 83% reduction in the predicted herd prevalence could be achieved with the combination of two control measures (Scenario: 2.18). The lowest predicted herd prevalence was 1% in a scenario combining all four control measures, including the eradication of hubs (Scenario: 4.8).

TABLE 2. Predicted median prevalence on 31st December 2012 for all scenarios presented in Manuscript III, including random voluntary eradication and eradication of hubs. The median herd prevalence without control measures was predicted to be 47% [42% - 52%].

Scenario ID	Scenario acronym				Predicted median prevalence in % (random eradication*) [90% prediction interval] (relative reduction)	Predicted median prevalence in % (eradication of hubs) [90% prediction interval] (relative reduction)
Single control measures						
1.7	Erad (1/year)				43 [38 - 49] (8%)	33 [27 - 37] (31%)
Combination of two control measures						
2.5	AB (50%)		Erad (1/year)		42 [26 - 56] (10%)	33 [17 - 45] (31%)
2.10	AB (100%)		Erad (1/year)		26 [11 - 40] (45%)	15[6 - 28] (68%)
2.13	ProbIT (50%)		Erad (1/year)		33 [28 - 39] (29%)	23 [19 - 27] (52%)
2.16	ProbIT (75%)		Erad (1/year)		28 [23 - 34] (41%)	18 [15 - 22] (62%)
2.17	MR (1/year)		Erad (1/year)		29 [25 - 34] (37%)	26 [22 - 30] (46%)
2.18	MR (4/year)		Erad (4/year)		21 [18 - 25] (55%)	8 [7 - 10] (83%)
Combination of three control measures						
3.3	AB (50%)	ProbIT (50%)	Erad (1/year)		32 [19 - 46] (31%)	21 [11 - 34] (54%)
3.6		ProbIT (75%)	Erad (1/year)		26 [15 - 38] (44%)	16 [8 - 27] (66%)
3.7		MR (1/year)	Erad (1/year)		29 [18 - 41] (39%)	24 [13 - 36] (48%)
3.8		MR (4/year)	Erad (4/year)		20 [11 - 31] (57%)	8 [3 - 14] (84%)
3.11	AB (100%)	ProbIT (50%)	Erad (1/year)		38 [32 - 43] (61%)	11 [4 - 20] (77%)
3.14		ProbIT (75%)	Erad (1/year)		15 [5 - 26] (68%)	8 [3 - 15] (83%)
3.15		MR (1/year)	Erad (1/year)		16 [8 - 28] (65%)	13 [5 - 22] (72%)
3.16		MR (4/year)	Erad (4/year)		10 [4 - 18] (79%)	3 [1 - 6] (94%)
3.17	ProbIT (50%)	MR (1/year)	Erad (1/year)		21 [17 - 25] (55%)	18 [14 - 22] (62%)
3.18		MR (4/year)	Erad (4/year)		15 [12 - 17] (69%)	5 [4 - 6] (90%)
3.19	ProbIT (75%)	MR (1/year)	Erad (1/year)		17 [14 - 21] (64%)	14 [11 - 17] (70%)
3.20		MR (4/year)	Erad (4/year)		12 [9 - 14] (75%)	4 [3 - 5] (92%)
Combination of four control measures						
4.1	AB (50%)	ProbIT (50%)	MR (1/year)	Erad (1/year)	20 [12 - 30] (56%)	17 [9 - 27] (64%)
4.2			MR (4/year)	Erad (4/year)	13 [8 - 22] (72%)	4 [2 - 8] (91%)
4.3	AB (50%)	ProbIT (75%)	MR (1/year)	Erad (1/year)	16 [9 - 24] (65%)	13 [7 - 20] (73%)
4.4			MR (4/year)	Erad (4/year)	11 [6 - 16] (77%)	3 [1 - 6] (93%)
4.5	AB (100%)	ProbIT (50%)	MR (1/year)	Erad (1/year)	11 [5 - 19] (77%)	9 [4 - 15] (82%)
4.6			MR (4/year)	Erad (4/year)	7 [3 - 13] (86%)	2 [1 - 4] (96%)
4.7	AB (100%)	ProbIT (75%)	MR (1/year)	Erad (1/year)	9 [5 - 15] (80%)	7 [3 - 12] (86%)
4.8			MR (4/year)	Erad (4/year)	6 [2 -10] (86%)	1 [1 - 3] (97%)

* Results presented in Manuscript III

4.3.1.3. HIGH HERD PREVALENCE IN 2006

Initialisation in 10,000 herds on 26th December 2006 led to a predicted median herd prevalence of 69% (90% prediction interval: 67% - 71%).

When the model was run with each implemented control measure separately, the median herd prevalence on 31st December 2012 was higher in all scenarios that started with high prevalence in 2006 compared to those with low herd prevalence in 2006 (Table Table 3a). The relative reduction for individual as well as for combinations of control measures decreased in almost all scenarios (exceptions: scenarios 1.1, 1.5, 2.3, 2.5). However, all control measures showed at least a small reduction in the modelled herd prevalence 6 years after initialisation of LA-MRSA, even when they were implemented individually (Tables 3a-d).

Combining two control measures led to a greater reduction in the modelled herd prevalence on 31st December 2012 (Table 3b). The lowest median herd prevalence modelled was for the combination of movement restrictions based on testing the herds for LA-MRSA four times per year and voluntary eradication (median: 24%, 90% prediction interval: 23% - 26%).

In the scenario where LA-MRSA was initialised with high herd prevalence in 2006, the combination of a reduction of high-risk antibiotic use in all herds, movement restrictions based on testing all herds for LA-MRSA four times per year and voluntary eradication of hubs (Scenario 3.16h) showed the lowest predicted median herd prevalence (median 12%, 90% prediction interval: 6% - 18%, Table 3c).

Combining all four control measures resulted in only a marginal additional reduction of herd prevalence on 31st December 2012 when starting with high herd prevalence in 2006, when compared to the lowest value reached in the combination of three control measures (Tables 3c-d). The lowest modelled herd prevalence was 8% (90% prediction interval: 3% - 10%) when a reduction in high-risk antibiotic use in all herds, a 75% reduction in the probability of indirect transmission via humans, movement restriction based on testing all herds for LA-MRSA four times per year and voluntary eradication were all combined (Scenario 4.8h).

TABLE 3A. Predicted median prevalence on 31st December 2012 for the default scenario presented in Manuscript III (low herd prevalence in 2006) and for a scenario with a high herd prevalence in 2006. All control measures were implemented individually.

Scenario ID	Scenario acronym	Predicted median herd prevalence in % on 31st December 2012 (low herd prevalence in 2006*) [90% prediction interval] (relative reduction)	Predicted median herd prevalence in % on 31st December 2012 (high herd prevalence in 2006) [90% prediction interval] (relative reduction)
No control measures			
0	Default	47 [42 - 52]	69 [67 - 71]
Single control measures			
1.1	AB (50%)	47 [30 - 61] (0%)	65 [55 - 74] (5%)
1.2	AB (100%)	29 [13 - 44] (38%)	60 [38 - 69] (13%)
1.3	ProbIT (50%)	37 [31 - 43] (21%)	67 [66 - 69] (2%)
1.4	ProbIT (75%)	31 [26 - 37] (33%)	66 [65 - 68] (4%)
1.5	MR (1/year)	47 [43 - 53] (0%)	62 [60 - 64] (10%)
1.6	MR (4/year)	37 [32 - 42] (22%)	63 [60 - 65] (9%)
1.7	Erad (1/year)	43 [38 - 49] (8%)	65 [63 - 67] (6%)
1.7h	Erad_hubs (1/year)	33 [27 - 37] (31%)	57 [54 - 59] (18%)

* Results presented in Table 5 in Manuscript III (except scenarios marked with h)

TABLE 3B. Predicted median prevalence on 31st December 2012 for the default scenario presented in Manuscript III (low herd prevalence in 2006) and for a scenario with a high herd prevalence in 2006. All combinations of two control measures were implemented.

Scenario ID	Scenario acronym		Predicted median herd prevalence in % on 31st December 2012 (low herd prevalence in 2006*) [90% prediction interval] (relative reduction)	Predicted median herd prevalence in % on 31st December 2012 (high herd prevalence in 2006) [90% prediction interval] (relative reduction)
No control measures				
0	Default		47 [42 - 52]	69 [67 - 71]
Combination of two control measures				
2.1	AB (50%)	ProbIT (50%)	36 [22 - 49] (24%)	64 [54 - 73] (8%)
2.2		ProbIt (75%)	30 [17 - 42] (37%)	63 [52 - 71] (9%)
2.3		MR (1/year)	47 [30 - 61] (0%)	57 [48 - 65] (17%)
2.4		MR (4/year)	35 [22 - 50] (25%)	59 [48 - 68] (15%)
2.5		Erad (1/year)	42 [26 - 56] (10%)	61 [51 - 70] (11%)
2.5h		Erad_hubs (1/year)	33 [17 - 45] (31%)	52 [41 - 62] (24%)
2.6	AB (100%)	ProbIT (50%)	22 [11 - 35] (54%)	56 [31 - 67] (19%)
2.7		ProbIT (75%)	15 [7 - 26] (68%)	58 [37 - 63] (16%)
2.8		MR (1/year)	30 [15 - 46] (36%)	50 [42 - 59] (27%)
2.9		MR (4/year)	21 [9 - 33] (56%)	52 [35 - 59] (25%)
2.10		Erad (1/year)	26 [11 - 40] (45%)	55 [36 - 66] (20%)
2.10h		Erad_hubs (1/year)	15 [6 - 28] (68%)	44 [12 - 52] (37%)
2.11	ProbIT (50%)	MR (1/year)	38 [32 - 43] (19%)	60 [58 - 62] (13%)
2.12		MR (4/year)	28 [23 - 33] (41%)	61 [59 - 63] (12%)
2.13		Erad (1/year)	33 [28 - 39] (29%)	62 [61 - 64] (9%)
2.13h		Erad_hubs (1/year)	23 [19 - 27] (52%)	53 [51 - 55] (23%)
2.14	ProbIT (75%)	MR (1/year)	32 [27 - 39] (31%)	58 [57 - 60] (15%)
2.15		MR (4/year)	23 [19 - 29] (50%)	59 [57 - 61] (14%)
2.16		Erad (1/year)	28 [23 - 34] (41%)	61 [59 - 63] (12%)
2.16h		Erad_hubs (1/year)	18 [15 - 22] (62%)	50 [48 - 52] (27%)
2.17	MR (1/year)	Erad (1/year)	29 [25 - 34] (37%)	57 [55 - 59] (18%)
2.17h		Erad_hubs (1/year)	26 [22 - 30] (46%)	52 [50 - 54] (24%)
2.18	MR (4/year)	Erad (4/year)	21 [18 - 25] (55%)	45 [43 - 47] (35%)
2.18h		Erad_hubs (4/year)	8 [7 - 10] (83%)	24 [23 - 26] (65%)

* Results presented in Table 5 in Manuscript III (except scenarios marked with h)

TABLE 3C. Predicted median prevalence on 31st December 2012 for the default scenario presented in Manuscript III (low herd prevalence in 2006) and for a scenario with a high herd prevalence in 2006. All combinations of three control measures were implemented.

Scenario ID	Scenario acronym			Predicted median herd prevalence in % on 31st December 2012 (low herd prevalence in 2006*) [90% prediction interval] (relative reduction)	Predicted median herd prevalence in % on 31st December 2012 (high herd prevalence in 2006) [90% prediction interval] (relative reduction)
No control measures					
0	Default			47 [42 - 52]	69 [67 - 71]
Combination of three control measures					
3.1	AB (50%)	ProbIT (50%)	MR (1/year)	36 [22 - 49] (23%)	55 [47 - 63] (20%)
3.2			MR (4/year)	26 [16 - 38] (44%)	56 [45 - 64] (19%)
3.3			Erad (1/year)	32 [19 - 46] (31%)	59 [48 - 68] (15%)
3.3h			Erad_hubs (1/year)	21 [11 - 34] (54%)	50 [40 - 57] (27%)
3.4		ProbIT (75%)	MR (1/year)	32 [20 - 43] (33%)	54 [44 - 61] (22%)
3.5			MR (4/year)	21 [13 - 33] (55%)	55 [44 - 64] (21%)
3.6			Erad (1/year)	26 [15 - 38] (44%)	57 [46 - 68] (18%)
3.6h			Erad_hubs (1/year)	16 [8 - 27] (66%)	43 [36 - 55] (37%)
3.7		MR (1/year)	Erad (1/year)	29 [18 - 41] (39%)	53 [43 - 61] (24%)
3.7h			Erad_hubs (1/year)	24 [13 - 36] (48%)	47 [38 - 57] (31%)
3.8		MR (4/year)	Erad (4/year)	20 [11 - 31] (57%)	42 [31 - 50] (40%)
3.8h			Erad_hubs (4/year)	8 [3 - 14] (84%)	20 [11 - 27] (71%)
3.9		ProbIT (50%)	MR (1/year)	22 [10 - 35] (53%)	50 [40 - 56] (27%)
3.10			MR (4/year)	15 [7 - 25] (68%)	52 [34 - 60] (28%)
3.11			Erad (1/year)	38 [32 - 43] (61%)	50 [37 - 64] (25%)
3.11h			Erad_hubs (1/year)	11 [4 - 20] (77%)	51 [11 - 46] (45%)
3.12	AB (100%)	ProbIT (75%)	MR (1/year)	19 [7-30] (59%)	38 [41 - 54] (30%)
3.13			MR (4/year)	13 [6 - 22] (72%)	48 [18 - 58] (31%)
3.14			Erad (1/year)	15 [5 - 26] (68%)	49 [32 - 58] (29%)
3.14h			Erad_hubs (1/year)	8 [3 - 15] (83%)	36 [27 - 45] (48%)
3.15		MR (1/year)	Erad (1/year)	16 [8 - 28] (65%)	46 [33 - 53] (34%)
3.15h			Erad_hubs (1/year)	13 [5 - 22] (72%)	38 [28 - 47] (44%)
3.16		MR (4/year)	Erad (4/year)	10 [4 - 18] (79%)	32 [19 - 43] (53%)
3.16h			Erad_hubs (4/year)	3 [1 - 6] (94%)	12 [6 - 18] (82%)
3.17	ProbIT (50%)	MR (1/year)	Erad (1/year)	21 [17 - 25] (55%)	54 [52 - 56] (22%)
3.17h			Erad_hubs (1/year)	18 [14 - 22] (62%)	48 [46 - 50] (30%)

Scenario ID	Scenario acronym			Predicted median herd prevalence in % on 31st December 2012 (low herd prevalence in 2006*) [90% prediction interval] (relative reduction)	Predicted median herd prevalence in % on 31st December 2012 (high herd prevalence in 2006) [90% prediction interval] (relative reduction)
3.18	ProbIT (75%)	MR (4/year)	Erad (4/year)	15 [12 - 17] (69%)	41 [39 - 43] (41%)
3.18h			Erad_hubs (4/year)	5 [4 - 6] (90%)	19 [18 - 21] (72%)
3.19		MR (1/year)	Erad (1/year)	17 [14 - 21] (64%)	52 [50 - 54] (24%)
3.19h			Erad_hubs (1/year)	14 [11 - 17] (70%)	46 [44 - 48] (33%)
3.20		MR (4/year)	Erad (4/year)	12 [9 - 14] (75%)	39 [37 - 41] (44%)
3.20h			Erad_hubs 4/year)	4 [3 - 5] (92%)	17 [16 - 18] (76%)

* Results presented in Table 5 in Manuscript III (except scenarios marked with h)

TABLE 3D. Predicted median prevalence on 31st December 2012 for the default scenario presented in Manuscript III (low herd prevalence in 2006) and for a scenario with a high herd prevalence in 2006. All combinations of four control measures were implemented.

Scenario ID	Scenario acronym				Predicted median herd prevalence in % on 31st December 2012 (low herd prevalence in 2006*) [90% prediction interval] (relative reduction)	Predicted median herd prevalence in % on 31st December 2012 (high herd prevalence in 2006) [90% prediction interval] (relative reduction)
No control measures						
0	Default				47 [42 - 52]	69 [67 - 71]
Combination of four control measures						
4.1	AB (50%)	ProbIT (50%)	MR (1/year)	Erad (1/year)	20 [12 - 30] (56%)	49 [41 - 57] (29%)
4.1h				Erad_hubs (1/year)	17 [9 - 27] (64%)	40 [35 - 52] (42%)
4.2			MR (4/year)	Erad (4/year)	13 [8 - 22] (72%)	36 [27 - 46] (47%)
4.2h				Erad_hubs (4/year)	4 [2 - 8] (91%)	14 (10 - 22] (80%)
4.3	AB (50%)	ProbIT (75%)	MR (1/year)	Erad (1/year)	16 [9 - 24] (65%)	48 [38 - 56] (31%)
4.3h				Erad_hubs (1/year)	13 [7 - 20] (73%)	41 [32 - 49] (41%)
4.4			MR (4/year)	Erad (4/year)	11 [6 - 16] (77%)	35 [27 - 43] (49%)
4.4h				Erad_hubs (4/year)	3 [1 - 6] (93%)	13 [9 - 19] (81%)
4.5	AB (100%)	ProbIT (50%)	MR (1/year)	Erad (1/year)	11 [5 - 19] (77%)	43 [29 - 50] (38%)
4.5h				Erad_hubs (1/year)	9 [4 - 14] (82%)	35 [27 - 43] (50%)
4.6			MR (4/year)	Erad (4/year)	7 [3 - 13] (86%)	30 [14 - 37] (57%)
4.6h				Erad_hubs (4/year)	2 [1 - 4] (96%)	9 [2 - 13] (87%)
4.7	AB (100%)	ProbIT (75%)	MR (1/year)	Erad (1/year)	9 [5 - 15] (80%)	37 [30 - 46] (46%)
4.7h				Erad_hubs (1/year)	7 [3 - 12] (86%)	34 [22 - 43] (51%)
4.8			MR (4/year)	Erad (4/year)	6 [2 -10] (86%)	28 [25 - 35] (59%)
4.8h				Erad_hubs (4/year)	1 [1 - 3] (97%)	9 [6 - 11] (88%)

* Results presented in Table 5 in Manuscript III (except scenarios marked with h)

4.3.1.4. HERDS AND MOVEMENTS AFFECTED BY CONTROL MEASURES

Without movement restrictions, 706,065 movements were modelled per iteration between 1st January 2006 and 31st December 2012. Between 0.2 - 12.3% of the movements were ignored (Tables 4a-d). The highest proportion of ignored movements was observed in the scenario with reduced use of high-risk antibiotics in 50% of the herds and movement restrictions based on testing all herds four times per year (Scenario 2.4, Table 4b). In contrast, the lowest number of ignored movements was found in the scenario where the use of high-risk antibiotics was stopped in all herds, the probability of effective transmission via indirect human contact was reduced by 75% and with movement restrictions based on testing all herds once per year (Scenario 3.12, Table 4c).

In scenarios with voluntary eradication, the median number of pigs sent to slaughter during the eradication process and the median number of herds using eradication were recorded and compared across the different simulated scenarios (Tables 4a-d). The number of pigs sent to slaughter during the eradication process was lowest in the scenario combining all four control measures (Scenario 4.7), with a median of 258,354 (90% prediction interval: 150,962 - 378,129), and highest in the scenario combining movement restrictions based on testing all herds four times per year and voluntary eradication (Scenario 2.18), with a median of 1,493,921 (90% prediction interval: 1,229,622 – 1,773,074). The same scenarios led to the lowest (highest) number of herds using eradication.

TABLE 4A. Number of ignored movements due to applied movement restrictions, pigs sent to slaughter during the eradication process and herds performing eradication in the simulated control scenarios between the implementation of control measures on 1st January 2007 and 31st December 2012, given as the median and 90% prediction intervals. All control measures were implemented individually.

Scenario ID	Scenario acronym	Ignored movements due to applied movement restrictions (proportion of ignored movements)	Number of pigs sent to slaughter during eradication process	Number of herds in eradication process
Single control measures				
1.1	AB (50%)	n.a.	n.a.	n.a.
1.2	AB (100%)	n.a.	n.a.	n.a.
1.3	ProbIT (50%)	n.a.	n.a.	n.a.
1.4	ProbIT (75%)	n.a.	n.a.	n.a.
1.5	MR (1/year)	3,015 [1,921 - 4,472] (0.4%)	n.a.	n.a.
1.6	MR (4/year)	81,543 [66,309 - 100,106] (11.5%)	n.a.	n.a.
1.7	Erad (1/year)	n.a.	846,744 [676,707 - 1,059,981]	423 [345 - 513]
1.7h	Erad_hubs (1/year)	n.a	1,392,703 [1,131,234 - 1,687,769]	501 [411 - 607]

TABLE 4B. Number of ignored movements due to applied movement restrictions, pigs sent to slaughter during the eradication process and herds performing eradication in the simulated control scenarios between the implementation of control measures on 1st January 2007 and 31st December 2012, given as the median and 90% prediction intervals. All combinations of two control measures are presented.

Scenario ID	Scenario acronym		Ignored movements due to applied movement restrictions (proportion of ignored movements)	Number of pigs sent to slaughter during eradication process	Number of herds in eradication process
Combination of two control measures					
2.1	AB (50%)	ProbIT (50%)	n.a.	n.a.	n.a.
2.2		ProbIT (75%)	n.a.	n.a.	n.a.
2.3		MR (1/year)	3,113 [1,855 - 5,077] (0.4%)	n.a.	n.a.
2.4		MR (4/year)	86,621 [57,471 - 114,874] (12.3%)	n.a.	n.a.
2.5		Erad (1/year)	n.a.	875,002 [575,435 - 1,178,923]	427 [276 - 582]
2.5h		Erad_hubs (1/year)	n.a.	1,435,321 [891,590 - 1,923,824]	516 [324 - 690]
2.6	AB (100%)	ProbIT (50%)	n.a.	n.a.	n.a.
2.7		ProbIT (75%)	n.a.	n.a.	n.a.
2.8		MR (1/year)	2,124 [849 - 3,914] (0.3%)	n.a.	n.a.
2.9		MR (4/year)	86,621 [57,471 - 114,874] (8.4%)	n.a.	n.a.
2.10		Erad (1/year)	n.a.	621,040 [341,496 - 905,089]	304 [175 - 442]
2.10h		Erad_hubs (1/year)	n.a.	923,040 [540,210 - 1,370,308]	332 [199 - 491]
2.11	ProbIT (50%)	MR (1/year)	2,373 [1,424 - 3,555] (0.3%)	n.a.	n.a.
2.12		MR (4/year)	64,286 [50,145 - 81,329] (9.1%)	n.a.	n.a.
2.13		Erad (1/year)	n.a.	690,257 [538,602 - 860,079]	340 [275 - 419]
2.13h		Erad_hubs (1/year)	n.a.	1,089,673 [881,169 - 1,333,437]	391 [321 - 480]
2.14	ProbIT (75%)	MR (1/year)	2,023 [1,168 - 3,336] (0.3%)	n.a.	n.a.
2.15		MR (4/year)	56,613 [43,676 - 73,504] (8.0%)	n.a.	n.a.
2.16		Erad (1/year)	n.a.	607,757 [481,599 - 788,955]	300 [242 - 390]
2.16h		Erad_hubs (1/year)	n.a.	945,772 [777,277 - 1,133,816]	344 [282 - 413]
2.17	MR (1/year)	Erad (1/year)	70,411 [56,788 - 86,836] (10.0%)	474,628 [378,392 - 593,597]	235 [191 - 284]
2.17h		Erad_hubs (1/year)	48,383 [38,788 - 60,329] (6.9%)	906,435 [761,574 - 1,094,137]	326 [279 - 386]
2.18	MR (4/year)	Erad (4/year)	60,199 [48,426 - 73,682] (8.5%)	1,493,921 [1,229,622 - 1,773,074]	714 [590 - 854]
2.18h		Erad_hubs (4/year)	15,542 [11,385 - 20,002] (2.2%)	1,705,509 [1,405,569 - 2,036,510]	657 [550 - 776]

TABLE 4C. Number of ignored movements due to applied movement restrictions, pigs sent to slaughter during the eradication process and herds performing eradication in the simulated control scenarios between the implementation of control measures on 1st January 2007 and 31st December 2012, given as the median and 90% prediction intervals. All combinations of three control measures are presented.

Scenario ID	Scenario acronym			Ignored movements due to applied movement restrictions (proportion of ignored movements)	Number of pigs sent to slaughter during eradication process	Number of herds in eradication process
Combination of three control measures						
3.1	AB (50%)	ProbIT (50%)	MR (1/year)	2,439 [1,215 - 3,830] (0.3%)	n.a.	n.a.
3.2			MR (4/year)	65,514 [48,426 - 73,682] (9.3%)	n.a.	n.a.
3.3			Erad (1/year)	n.a.	703,942 [451,846 - 962,944]	343 [218 - 484]
3.3h			Erad_hub (1/year)	n.a.	1,068,327 [699,305 - 1,485,979]	386 [259 - 538]
3.4		ProbIT (75%)	MR (1/year)	2,104 [1,089 - 3,492] (0.3%)	n.a.	n.a.
3.5			MR (4/year)	57,718 [39,346 - 80,695] (8.2%)	n.a.	n.a.
3.6			Erad (1/year)	n.a.	595,474 [401,239 - 855,955]	289 [197 - 418]
3.6h			Erad_hub (1/year)	n.a.	915,637 [609,864 - 1,294,360]	331 [218 - 469]
3.7		MR (1/year)	Erad (1/year)	73,423 [49,257 - 100,473] (10.4%)	490,278 [323,939 - 681,882]	239 [158 - 333]
3.7h			Erad_hub (1/year)	51,007 [29,372 - 71,959] (7.2%)	914,229 [593,879 - 1,235,039]	329 [216 - 451]
3.8		MR (4/year)	Erad (4/year)	62,188 [41,887 - 86,685] (8.8%)	1,485,901 [1,009,896 - 2,046,549]	694 [474 - 977]
3.8h			Erad_hub (4/year)	18,060 [10,483 - 26,463] (2.6%)	1,678,637 [1,130,378 - 2,392,509]	634 [432 - 903]
3.9	AB (100%)	ProbIT (50%)	MR (1/year)	1,645 [628 - 2,908] (0.2%)	n.a.	n.a.
3.10			MR (4/year)	47,935 [26,429 - 64,351] (6.8%)	n.a.	n.a.
3.11			Erad (1/year)	n.a.	487,799 [291,739 - 721,247]	238 [148 - 341]
3.11h			Erad_hub (1/year)	n.a.	768,650 [460,994 - 1,119,151]	279 [175 - 403]
3.12		ProbIT (75%)	MR (1/year)	1,490 [461 - 2,844] (0.2%)	n.a.	n.a.
3.13			MR (4/year)	42,884 [24,843 - 64,351] (6.1%)	n.a.	n.a.
3.14			Erad (1/year)	n.a.	453,890 [254,482 - 671,290]	220 [131 - 326]
3.14h			Erad_hub (1/year)	n.a.	660,042 [378,122 - 968,699]	242 [144 - 349]
3.15		MR (1/year)	Erad (1/year)	52,738 [30,370 - 78,672] (7.5%)	353,194 [207,753 - 518,182]	171 [106 - 246]
3.15h			Erad_hub (1/year)	35,905 [18,739 - 56,234] (5.1%)	650,116 [395,937 - 964,670]	236 [147 - 345]
3.16		MR (4/year)	Erad (4/year)	41,987 [23,557 - 65,946] (5.9%)	1,017,999 [591,626 - 1,551,999]	473 [290 - 720]
3.16h			Erad_hub (4/year)	11,757 [5,598 - 19,258] (1.7%)	1,158,809 [717,719 - 1,698,305]	446 [290 - 636]
3.17	ProbIT (50%)	MR (1/year)	Erad (1/year)	56,265 [44,271 - 69,209] (8.0%)	385,045 [298,923 - 481,688]	187 [150 - 231]
3.17h			Erad_hub (1/year)	37,567 [228,713 - 48,852] (5.3%)	720,856 [517,480 - 777,598]	262 [217 - 317]
3.18		MR (4/year)	Erad (4/year)	46,515 [36,798 - 58,021] (6.6%)	1,194,142 [972,157 - 1,425,783]	572 [473 - 675]
3.18h			Erad_hub (4/year)	11,251 [8,502 - 14,828] (1.6%)	1,367,455 [1,146,626 - 1,630,613]	533 [457 - 631]
3.19	ProbIT (75%)	MR (1/year)	Erad (1/year)	48,930 [38,834 - 62,110] (6.9%)	337,089 [249,895 - 435,706]	166 [128 - 203]
3.19h			Erad_hub (1/year)	32,473 [24,459 - 41,334] (4.6%)	635,751 [517,480 - 777,598]	231 [192 - 278]
3.20		MR (4/year)	Erad (4/year)	41,767 [32,625 - 52,761] (5.9%)	1,071,833 [876,921 - 1,314,096]	510 [425 - 624]
3.20h			Erad_hub (4/year)	9,844 [7,434 - 13,022] (1.4%)	1,236,589 [1,057,979 - 1,509,640]	484 [417 - 580]

TABLE 4D. Number of ignored movements due to applied movement restrictions, pigs sent to slaughter during the eradication process and herds performing eradication in the simulated control scenarios between the implementation of control measures on 1st January 2007 and 31st December 2012, given as the median and 90% prediction intervals. All combinations of four control measures are presented.

Scenario ID	Scenario acronym				Ignored movements due to applied movement restrictions (proportion of ignored movements)	Number of pigs sent to slaughter during eradication process	Number of herds in eradication process
Combination of four control measures							
4.1	AB (50%)	ProbIT (50%)	MR (1/year)	Erad (1/year)	57,012 [39,603 - 77,580] (8.1%)	382,819 [248,209 - 525,817]	186 [126 - 256]
4.1h				Erad_hubs (1/year)	39,472 [25,264 - 54,344] (5.6%)	718,338 [480,116 - 975,919]	257 [176 - 350]
4.2			MR (4/year)	Erad (4/year)	47,383 [33,330 - 65,429] (6.7%)	1,155,733 [831,920 - 1,616,957]	545 [389 - 771]
4.2h				Erad_hubs (4/year)	13,385 [8,088 - 19,616] (1.9%)	1,333,259 [921,941 - 1,844,933]	511 [368 - 698]
4.3	AB (50%)	ProbIT (75%)	MR (1/year)	Erad (1/year)	49,786 [32,631 - 66,592] (7.1%)	334,818 [213,268 - 469,031]	162 [105 - 228]
4.3h				Erad_hubs (1/year)	32,773 [22,894 - 46,183] (4.6%)	623,546 [430,038 - 842,242]	224 [162 - 303]
4.4			MR (4/year)	Erad (4/year)	41,848 [27,987 - 56,454] (5.9%)	1,033,152 [727,110 - 1,419,543]	484 [335 - 672]
4.4h				Erad_hubs (4/year)	11,018 [6,734 - 16,165] (1.6%)	1,175,029 [812,804 - 1,579,280]	454 [328 - 603]
4.5	AB (100%)	ProbIT (50%)	MR (1/year)	Erad (1/year)	41,824 [25,129 - 62,197] (5.9%)	272,576 [168,738 - 416,836]	135 [85 - 199]
4.5h				Erad_hubs (1/year)	27,732 [16,103 - 43,470] (3.9%)	521,689 [334,068 - 752,720]	191 [126 - 267]
4.6			MR (4/year)	Erad (4/year)	34,017 [20,958 - 49,912] (4.8%)	819,128 [522,736 - 1,215,928]	392 [254 - 564]
4.6h				Erad_hubs (4/year)	9,557 [5,056 - 15,284] (1.4%)	985,815 [635,082 - 1,367,362]	382 [260 - 523]
4.7	AB (100%)	ProbIT (75%)	MR (1/year)	Erad (1/year)	38,971 [23,765 - 56,620] (5.5%)	258,354 [150,962 - 378,129]	127 [78 - 179]
4.7h				Erad_hubs (1/year)	24,957 [13,904 - 37,381] (3.5%)	470,197 [296,766 - 663,725]	170 [112 - 236]
4.8			MR (4/year)	Erad (4/year)	31,107 [18,119 - 45,369] (4.4%)	772,334 [463,941 - 1,086,570]	362 [227 - 500]
4.8h				Erad_hubs (4/year)	8,380 [4,364 - 13,618] (1.2%)	895,348 [575,703 - 1,225,681}	348 [231 - 472]

4.3.2. Discussion

In Manuscript III, the model presented in Manuscript II was enhanced by four potential control measures: (1) a reduced number of herds using high-risk antibiotics, (2) increased biosecurity to reduce indirect transmissions via humans, (3) movement restrictions prohibiting trade from LA-MRSA-positive to negative herds and (4) voluntary eradication in 7.5% (5%) of the breeding and multiplier herds (other herd types). We aimed to investigate how these control measures and their combinations could have influenced the spread of LA-MRSA in the study period 2006 to 2015 if they had been implemented in 2007. We could show that almost all control measures led to reductions in the predicted median herd prevalence compared to the default scenario if they had been implemented in 2007. Even implementing control measures in 2010, when the predicted herd prevalence was higher than 2007, led to similar (slightly lower) reduction rates (Manuscript III). The predicted herd prevalence in 2006 was far below the current prevalence of 88% in Danish pig herds. We therefore increased LA-MRSA initialisation in 2006 to mimic a scenario with high herd prevalence prior to the start of control measures (Chapter 4.3.1). Reduction rates were smaller compared to the default scenario presented in Manuscript III, irrespective of which control measure or combination was tested. However, a reduction in the predicted median herd prevalence could be shown for all control measures and combinations. Therefore, intense and combined control measures beginning in the current situation in Danish herds might still lead to a clear reduction in LA-MRSA herd prevalence. However, this would require considerable and rigorous efforts, for which economic consequences are yet to be estimated.

The reduction in herds using high-risk antibiotics could be interpreted as a reduction in the within-herd transmission rates. Each intervention leading to lower within-herd prevalence might also reduce the spread of LA-MRSA among pig herds. However, to the best of my knowledge, it has been shown that only a decrease in antimicrobial consumption leads to a decrease in within-herd prevalence[85].

Running all control scenarios until the end of 2015 showed that implemented control measures only slowed down the spread of LA-MRSA between pig herds when implemented individually or in combinations of two or three control measures (Chapter 4.3.1.1). None of these scenarios were able to reduce the herd prevalence after initialisation. However, combining all four control measures kept the predicted LA-MRSA herd prevalence at an almost constant level between 2012 and 2015. This result indicates that only considerable rigorous efforts might help to reduce the present herd prevalence of LA-MRSA in swine herds in Denmark.

In Chapter 4.3.1.1, we investigated how effective eradication of hubs would be compared to randomly chosen herds in the voluntary eradication programme. Hubs (defined as herds with the largest out-going contact chains of all herds) that tested positive in the simulation were chosen to start the eradication process. This approach led to a reduction in the predicted herd prevalence compared to the scenarios with randomly chosen herds in the eradication process. Therefore, prioritising herds with a higher potential to spread disease via pig movements could be an option to increase the effects of an eradication programme.

Starting control measures when the prevalence was assumed to be at a high level led to at least slight reductions in the predicted herd prevalence in all implemented control scenarios (Chapter 4.3.1.3). However, these reductions were clearly smaller compared to starting control measures at a low prevalence. However, the effects were smaller when implementing combinations of three or four control measures. In addition, scenarios in which herds with a higher potential were prioritised for eradication showed a high potential of limiting the spread of LA-MRSA. This highlights the need of rigorous efforts, especially when implementing control measures when the prevalence is already at a high level.

Movement restrictions and voluntary eradication were shown to be effective control measures, especially when combined with other implemented control options. However, both of these measures would have an effect on the pig industry (Chapter 4.3.1.4). In the case of movement restrictions, trade prohibition from LA-MRSA-positive to negative herds might lead to new trade connections and to an increased export of pigs. Eradication implies that pigs must be sent to slaughter and that herds should be re-stocked with LA-MRSA-negative pigs. The number of pigs and herds varied depending on the control scenario, and this must be taken into account when implementing a national action plan. Besides economic losses from sending pigs to slaughter or culling due to eradication, there might also be a loss of breeding stock, and it may not be possible to compensate for this economically.

Simplified versions of the control measures were implemented. For example, we assumed that all herds were tested on the same day, which is not realistic. Therefore, the effects of control measures might differ if another paradigm were to be used.

5. General discussion

This thesis aimed to provide a better insight into the spread of LA-MRSA among pig herds, quantify the driving mechanisms of LA-MRSA spread between herds and study the effect of measures to control LA-MRSA spread. All analyses were based on the structure of pig herds and the movements of pigs between herds registered in Denmark between 1st January 2006 and 31st December 2015. Network analysis tools were used to describe the dataset used, to investigate contact patterns among pig herds and to identify potential hubs for disease spread. A simulation model was developed to mimic the spread of LA-MRSA among Danish pig herds and to investigate the routes of transmission. Four potential control options were retrospectively tested for their potential to control the spread of LA-MRSA, both individually and combined.

In general, this thesis illustrates how herd information data, movement data, information on LA-MRSA prevalence in Denmark and information on LA-MRSA in general could be combined to provide a better insight into the mechanisms of disease spread and the potential for control measures to reduce spread among pig herds. The results could be used to adapt the current Danish action plan, but could also be used in other regions/countries with LA-MRSA in the pig population. The results of this thesis might present realistic expectations of an achievable reduction in herd prevalence with either implemented or planned control measures, independent of the current LA-MRSA status of the considered region.

5.1. Limitations

All analyses were based on herd information and pig movements registered in the Central Husbandry Register (CHR) in Denmark between 1st January 2006 and 31st December 2015.

A central discussion point in terms of the CHR is the lack of herd-type definitions registered by the farmers. In particular, the combination of breeding and multiplier herds posed challenges during the analysis. Breeding herds do not usually have incoming movements; they produce and sell purebred breeding stock. Multiplier herds produce and sell hybrids (in addition to purebred breeding stock) and could have incoming pig movements. Being able to separate these holding types would have led to more detailed information. The impact of initialising LA-MRSA in breeding herds or multiplier herds on the

model outcome could have been evaluated in the model. This might have led to a better understanding of the role of breeding herds in the initialisation process, as well as in the spread of LA-MRSA in general.

Information based on the registered herd type must be used with care, as discussed above. However, to overcome this, it may be possible to re-categorise the herds using a recently developed algorithm for automatic classification of farms and traders in the pig production chain, based on the farm's trade pattern[86]. In the case of eradicating hubs, re-categorisation might have led to even higher rates of reduction of the predicted herd prevalence. However, when implementing control measures in reality, categorising all herds according to the herd's trade pattern might pose additional challenges.

There were no sows, weaners or finishers registered for some of the herds. We therefore estimated values based on herds of a similar type (for a detailed description, see Supplemental Information in Manuscript II). When using this information for research, updated herd information would help to avoid additional uncertainties, including those faced when estimating missing information.

Model predictions are limited by the assumed model structure and the data used to parameterise the model. There is a lot of information about the structure of Danish pig production, yet the size of registered herds might not be up-to-date in all cases. Data on registered pig movements are generally of good quality, but the age group of the pigs that were moved (sows, weaners, finishers) is not registered. In general, there is still a lack of knowledge about different transmission routes between pig herds. Obtaining more precise information on pig herds and the ages of pigs moved would increase the data quality – not only for input in the presented model, but for scientific use in general. Additionally, LA-MRSA could be transmitted among different animal species or individual animals, between humans and animals, or other potential sources. These transmission routes and their importance in the spread of LA-MRSA must be studied in detail. This would allow us to model these processes more realistically, rather than initiating random introductions in herds, as carried out in 2006 and 2009 for instance (Manuscript II and III), thus allowing more precise model predictions. As such, new control measures could be specified based on the gained knowledge, which could be tested in the simulation model.

6. CONCLUSIONS

The aim of the network analysis was to identify contact patterns between pig herds and hubs for disease spread. The following can be concluded:

- Although most contact between different holding types follow the production pyramid, many horizontal connections also exist.
- There is considerable variation among herds of the same holding type in terms of their loyalty patterns. Therefore, general statements could not be made for holding types.
- There are substantial variations among pig herds of the same holding types in terms of the size of out-going contact chains. Therefore, each herd must be categorised independently of the registered holding type in order to estimate its potential as a hub for disease spread.

To understand and assess the impact of potential routes of LA-MRSA transmission among Danish swine herds, a simulation model was developed. Based on the assumptions made to mimic the transmission mechanisms, the underlying parameters and the different scenarios simulated, it can be concluded that:

- Breeding and multiplier herds played a key role when simulating the initialisation of LA-MRSA. This highlights their potential as hubs for disease spread, because they are located at the top of the production pyramid. Avoiding LA-MRSA introduction to breeding and multiplier herds might therefore have led to a reduction in the spread of LA-MRSA.
- Transmission via animal movements alone was not sufficient to mimic the development of the LA-MRSA herd prevalence observed in Denmark during the considered study period (2006 to 2015). However, movement of live animals was a driving force of LA-MRSA spread among herds.
- The frequency of indirect transmission via humans, as well as the effectiveness of indirect contact between herds via humans greatly influenced the model results. The higher the number of contacts; the greater the spread among pig herds and vice versa.
- The between-herd prevalence was highly correlated with the within-herd prevalence. Reducing the within-herd prevalence would therefore be expected to aid in controlling LA-MRSA.

The effects of different control strategies on the spread of LA-MRSA among Danish pig herds was investigated and led to the following conclusions:

- Reducing the proportion of herds that use high-risk antibiotics would have reduced the within-herd transmission and prevalence, which would have reduced the predicted between-herd prevalence when control was initiated in 2007 or 2010.
- Increasing biosecurity by reducing the probability of indirect transmission via humans would also have reduced the between-herd prevalence.
- Imposing movement restrictions alone based on testing all herds once per year would not have had an effect on the predicted herd prevalence. However, testing all herds four times per year followed by trade prohibition from LA-MRSA-positive to negative herds showed a clear reduction in the predicted herd prevalence.
- Eradication based on randomly chosen herds was not as efficient as targeted selection of herds based on their potential to spread the disease from out-going animal movements.
- Combining control measures showed an additive effect and led to the highest rate of reduction when all control measures were combined and implemented in the most intense version.
- Starting control measures in 2007 with a high prevalence led to a lower reduction in herd prevalence compared to starting at a low prevalence. However, even with a high prevalence at the start of control, clear reductions in the between-herd prevalence could be achieved.

7. PERSPECTIVES

During the modelling process, we identified knowledge gaps relating to some model parameters, such as quantification of the effectiveness of indirect contact between pig herds, and some model processes, for instance related to environmental transmissions. Furthermore, the transmission rates within the herds were shown to strongly influence the model outcome. However, little information is available on the effects of interventions to lower the within-herd prevalence. More precise information gained from experimental studies could be used to update the model parameters and to adapt the model processes, leading to more precise predictions.

When setting up control measures, effective surveillance strategies must also be implemented to assess the performance of control measures and to identify LA-MRSA-positive herds for eradication. Surveillance options could be added to the existing model to evaluate the timeliness and effectiveness of re-introductions or new introductions of LA-MRSA in the pig population. This could be of special interest after reducing the herd prevalence to a specific pre-defined level, or when a decision must be made to terminate control measures.

The model could also be used to assess the impact of control strategies that have been proven to reduce within-herd prevalence on the between-herd spread of LA-MRSA.

The model was developed to provide a better insight into the spread of LA-MRSA among pig herds and to evaluate potential control measures. However, it could be adapted to other diseases that spread via pig movements among pig herds. The transmission parameters could be easily updated. Modules for other transmission pathways could be switched on/off depending on the pathogen under consideration. Additionally, new modules could be implemented and added to the original model.

The current model ran for a retrospective study period. Pig movements could also be simulated based on determined movement patterns in order to mimic future study periods. However, it is uncertain how farmers would react to control measures: predicting more exports or adjusted trade connections would add more uncertainty to the modelled results and would therefore make the predictions less reliable.

Finally, the model could be enhanced by economic analysis in order to assess the cost-effectiveness of the implemented control measures.

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9. Appendix

9.1. Supplementary Information for Manuscript I

9.1.1. S1 File: Size of holdings

Table 1. Size of holdings of active Danish pig holdings. Descriptive statistics of the holding sizes of active Danish pig holdings (holdings that at least once send or receive pigs to or from another holding) between 1 Jan 2006 and 31 Dec 2015 in Denmark. If no sows, finishers and weaners were registered, the total holding size was set to "not available".

	Number of active holdings	Minimum	1st Quantile	Median	Mean	3rd Quantile	Maximum	Not available
2006	12,814	1	252	816	1,227	1,737	15,750	880
2007	11,920	1	300	823	1,254	1,700	29,940	398
2008	10,886	1	395	1,000	1,362	1,900	29,940	390
2009	9,816	1	440	1,086	1,474	2,000	29,940	392
2010	9,270	1	500	1,200	1,566	2,121	29,940	374
2011	8,875	1	520	1,200	1,598	2,230	23,950	342
2012	8,502	1	600	1,300	1,668	2,300	21,000	561
2013	8,370	1	650	1,400	1,742	2,400	21,000	952
2014	8,366	1	700	1,500	1,796	2,430	21,000	1,296
2015	7,835	1	600	1,410	1,813	2,500	19,620	536

Table 2. Proportion of active holdings by holding size. Proportion of holdings active between 1 Jan 2006 and 31 Dec 2015 in Denmark, categorized by the size of holding.

Herdsize	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
0	6.87	3.34	3.58	3.99	4.03	3.85	6.60	11.37	15.49	6.84
0 - 1,000	53.71	56.08	50.35	46.38	43.39	41.72	38.44	33.91	30.67	36.23
1,001 - 2,000	21.62	23.00	25.03	25.96	26.67	26.63	26.28	25.89	25.26	25.45
2,001 - 3,000	9.42	10.19	12.32	13.47	14.36	15.13	15.28	15.33	15.14	15.33
3,001 - 4,000	4.39	3.47	4.77	5.45	6.08	6.58	7.00	7.00	6.97	7.76
4,001- 5,000	1.76	1.61	1.78	2.25	2.47	2.96	3.16	3.23	3.18	3.87
5,001 - 6,000	0.99	0.74	0.92	1.10	1.39	1.58	1.53	1.51	1.53	1.83
6,001 - 7,000	0.66	0.36	0.42	0.47	0.59	0.70	0.69	0.73	0.72	1.03
7,001 - 8,000	0.23	0.26	0.23	0.25	0.29	0.34	0.47	0.48	0.48	0.65
8,001 - 9,000	0.09	0.18	0.13	0.14	0.17	0.12	0.15	0.16	0.16	0.32
9,001 - 10,000	0.07	0.12	0.06	0.10	0.12	0.07	0.09	0.10	0.10	0.27
10,001 - 11,000	0.07	0.09	0.10	0.08	0.09	0.11	0.12	0.12	0.12	0.14
11,001 - 12,000	0.05	0.11	0.05	0.04	0.05	0.08	0.09	0.10	0.10	0.13
12,001 - 13,000	0.03	0.10	0.05	0.05	0.05	0.01	0.02	0.02	0.02	0.08
13,001 - 14,000		0.06	0.03	0.02	0.02	0.05	0.04	0.04	0.04	0.01
14,001 - 15,000	0.02	0.08	0.02	0.03	0.04	0.01	0.01	0.01	0.01	0.03
15,001 - 16,000	0.02	0.02				0.01				
16,001 - 17,000		0.06	0.04	0.03	0.01	0.01	0.01	0.01	0.01	
17,001 - 18,000		0.04	0.01	0.02	0.01					0.01
18,001 - 19,000		0.03	0.03	0.02						
19,001 - 20,000		0.01	0.03	0.03	0.03	0.01				0.03
20,001 - 21,000		0.01	0.02	0.02	0.02	0.01	0.01	0.01	0.01	
21,001 - 22,000										
22,001 - 23,000			0.01							

Herdsize	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
23,001 - 24,000			0.02	0.02	0.02	0.01				
24,001 - 25,000		0.01	0.01	0.03	0.04					
25,001 - 26,000		0.01								
26,001 - 27,000		0.01	0.01	0.01	0.01					
27,001 - 28,000										
28,001 - 29,000				0.01	0.01					
29,001 - 30,000		0.01	0.01	0.01	0.01					

Figure 1. Holding sizes by holding type. Median (solid line) and average (dashed line) of holding sizes for (a) breeding sites, (b) production sites, (c) hobby sites, (d) transit sites, and (e) miscellaneous sites. Coloured areas represent the range between 1st and 3rd quantile. Values for holding types not shown in this figure are not representative and shown in Table 3-8.

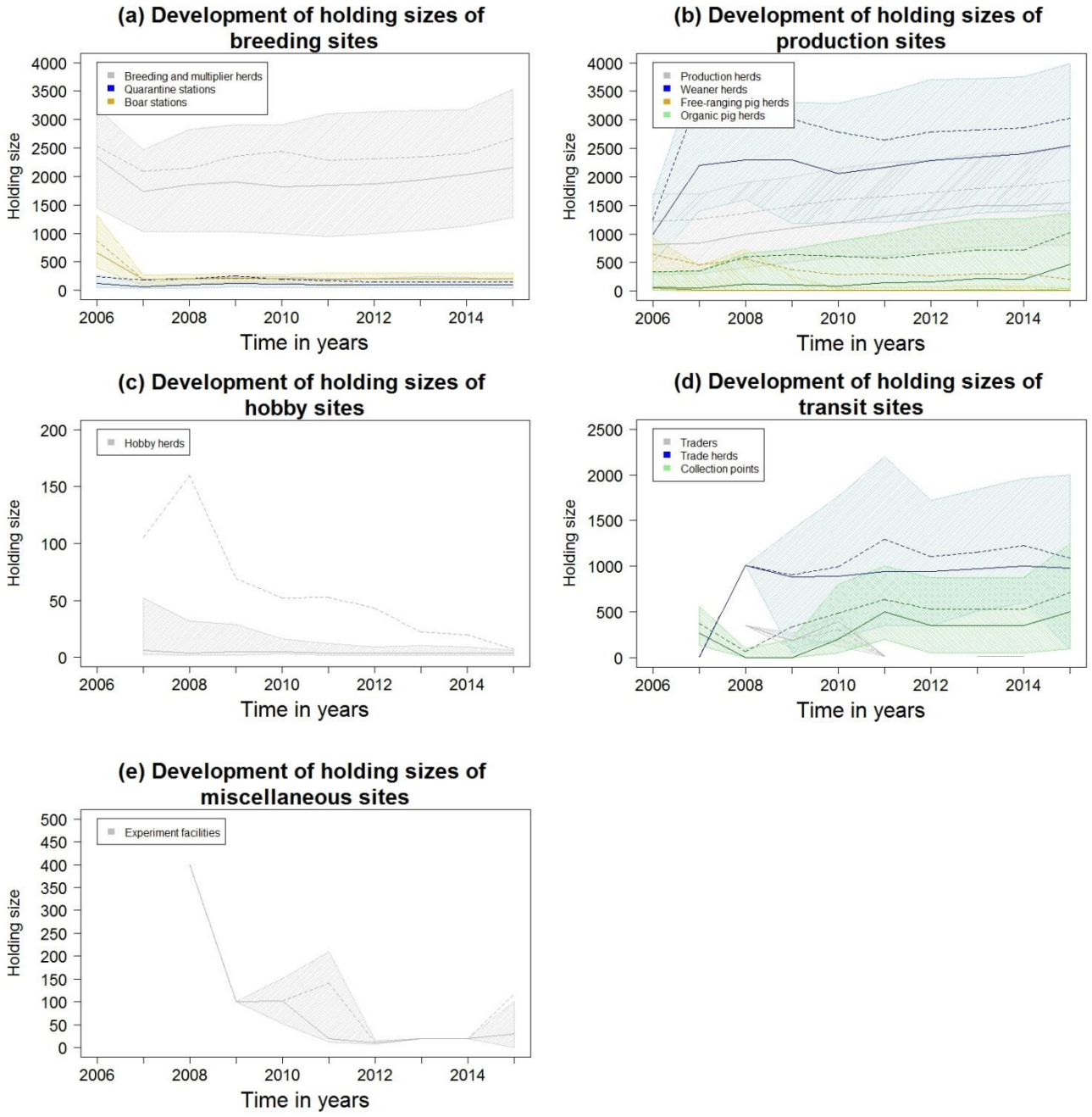


Table 3. Descriptive summaries of holding sizes for breeding sites.

		Number of active holdings	Minimum	1st quantile	Median	Mean	3rd quantile	Maximum	Not available
Breeding and multiplier herds	2006	278	6	1,450	2,330	2,532	3,204	12,580	18
	2007	261	2	1,028	1,740	2,097	2,465	18,500	2
	2008	252	2	1,028	1,860	2,141	2,828	10,950	1
	2009	263	2	1,026	1,900	2,354	2,910	24,890	2
	2010	270	2	1,000	1,825	2,442	2,910	24,890	1
	2011	276	2	950	1,846	2,291	3,102	19,200	0
	2012	272	1	1,000	1,870	2,308	3,138	10,050	4
	2013	271	1	1,050	1,940	2,346	3,153	10,050	12
	2014	260	1	1,132	2,040	2,404	3,170	10,050	16
	2015	234	1	1,280	2,150	2,663	3,530	11,950	1
Quarantine stations	2006	71	1	50	125	240	267	2,005	11
	2007	75	1	40	60	178	125	2,960	4
	2008	62	1	33	100	209	205	3,200	2
	2009	57	3	60	125	250	240	3,200	6
	2010	48	3	50	105	192	243	1,250	4
	2011	39	3	45	98	172	205	2,000	3
	2012	46	10	48	100	145	200	700	5
	2013	41	10	45	95	147	240	700	4
	2014	36	1	47	100	146	230	700	1
	2015	44	3	40	95	150	160	600	3
Boar stations	2006	18	52	396	660	881	1,320	2,046	3
	2007	17	20	85	197	189	271	393	0
	2008	16	26	108	211	207	278	393	0
	2009	15	26	145	215	210	285	393	0
	2010	16	26	140	206	206	278	393	0
	2011	16	25	72	200	194	299	405	0
	2012	17	26	95	202	200	298	405	0
	2013	17	26	95	202	200	298	405	0
	2014	17	26	95	202	200	298	405	0
	2015	17	26	95	202	203	298	405	0

Table 4. Descriptive summaries of holding sizes for production sites.

		Number of active holdings	Minimum	1st quantile	Median	Mean	3rd quantile	Maximum	Not available
Production herds	2006	11733	1	287	817	1,222	1,700	15,750	548
	2007	10867	1	300	845	1,253	1,700	29,940	193
	2008	9902	1	410	1,000	1,361	1,900	29,940	195
	2009	8691	1	500	1,100	1,482	2,000	29,940	196
	2010	7940	1	600	1,200	1,601	2,150	29,940	161
	2011	7538	1	614	1,300	1,647	2,250	23,950	110
	2012	7090	1	700	1,400	1,723	2,303	21,000	200
	2013	6819	1	767	1,500	1,792	2,400	21,000	355
	2014	6623	1	800	1,500	1,844	2,440	21,000	444
	2015	6230	1	800	1,550	1,938	2,510	19,620	110
Weaner herds	2006	110	30	500	1,000	1,241	1,650	5,080	47
	2007	105	30	1,400	2,200	3,431	4,000	26,600	0
	2008	106	1	1,600	2,300	3,377	3,625	26,600	2
	2009	171	98	1,188	2,300	3,018	3,300	26,600	0
	2010	241	12	1,200	2,062	2,789	3,285	26,600	1
	2011	240	1	1,210	2,162	2,648	3,475	12,400	2
	2012	227	3	1,249	2,288	2,782	3,706	12,400	3
	2013	224	3	1,362	2,345	2,821	3,726	12,400	6
	2014	223	3	1,407	2,400	2,860	3,751	12,400	12
	2015	201	7	1,400	2,545	3,028	3,989	13,000	2

		Number of active holdings	Minimum	1st quantile	Median	Mean	3rd quantile	Maximum	Not available
Free-ranging pig herds	2006	325	1	10	52	644	936	8,000	45
	2007	279	1	4	20	458	450	10,330	26
	2008	189	1	5	18	559	729	8,255	22
	2009	190	1	5	18	378	250	5,495	25
	2010	157	1	4	11	291	54	5,495	19
	2011	145	1	4	12	304	60	5,495	19
	2012	141	1	4	14	262	72	3,500	35
	2013	153	1	4	14	300	83	3,500	66
	2014	169	1	4	13	300	83	3,500	82
	2015	164	1	3	10	204	55	4,350	36
Organic herds	2006	76	1	14	65	338	331	3,080	9
	2007	79	1	10	45	343	350	3,835	6
	2008	117	1	10	118	595	658	10,070	5
	2009	106	1	15	115	640	738	6,297	4
	2010	99	1	12	83	610	875	6,297	6
	2011	93	2	12	140	573	1,000	3,150	4
	2012	90	1	13	159	649	1,162	5,495	6
	2013	87	1	22	223	722	1,261	5,495	11
	2014	90	3	20	210	717	1,272	5,495	15
	2015	88	1	41	470	1,021	1,360	8,490	5

Table 5. Descriptive summaries of holding sizes for hobby sites.

		Number of active holdings	Minimum	1st quantile	Median	Mean	3rd quantile	Maximum	Not available
Hobby herds	2006	69	NA	NA	NA	NA	NA	NA	69
	2007	101	1	3	6	105	52	1,600	45
	2008	110	1	2	4	160	32	3,600	43
	2009	196	1	2	5	69	29	2,150	55
	2010	358	1	3	5	52	16	1,585	79
	2011	391	1	2	4	53	12	1,810	104
	2012	454	1	2	4	43	9	3,027	178
	2013	561	1	2	4	22	10	830	335
	2014	721	1	2	4	20	9	830	531
	2015	521	1	2	4	7	6	281	96
Pets	2006	5	NA	NA	NA	NA	NA	NA	5
	2007	2	NA	NA	NA	NA	NA	NA	2
	2008	1	NA	NA	NA	NA	NA	NA	1
	2009	1	NA	NA	NA	NA	NA	NA	1
	2010	2	500	688	875	875	1,062	1,250	0
	2011	2	350	350	350	350	350	350	1
	2012	1	NA	NA	NA	NA	NA	NA	1
	2013	3	NA	NA	NA	NA	NA	NA	3
	2014	10	NA	NA	NA	NA	NA	NA	10
	2015	11	1	1	1	4	3	13	6

		Number of active holdings	Minimum	1st quantile	Median	Mean	3rd quantile	Maximum	Not available
Wild boar herds	2006	0	NA	NA	NA	NA	NA	NA	0
	2007	2	NA	NA	NA	NA	NA	NA	2
	2008	1	NA	NA	NA	NA	NA	NA	1
	2009	1	NA	NA	NA	NA	NA	NA	1
	2010	1	NA	NA	NA	NA	NA	NA	1
	2011	1	NA	NA	NA	NA	NA	NA	1
	2012	15	4	4	4	4	4	4	14
	2013	8	NA	NA	NA	NA	NA	NA	8
	2014	8	NA	NA	NA	NA	NA	NA	8
	2015	11	2	4	13	25	48	72	1
Organic wild boar herds	2006	0	NA	NA	NA	NA	NA	NA	0
	2007	0	NA	NA	NA	NA	NA	NA	0
	2008	0	NA	NA	NA	NA	NA	NA	0
	2009	0	NA	NA	NA	NA	NA	NA	0
	2010	0	NA	NA	NA	NA	NA	NA	0
	2011	0	NA	NA	NA	NA	NA	NA	0
	2012	0	NA	NA	NA	NA	NA	NA	0
	2013	0	NA	NA	NA	NA	NA	NA	0
	2014	0	NA	NA	NA	NA	NA	NA	0
	2015	1	8	8	8	8	8	8	0

Table 6. Descriptive summaries of holding sizes for transit sites.

		Number of active holdings	Minimum	1st quantile	Median	Mean	3rd quantile	Maximum	Not available
Traders	2006	7	NA	NA	NA	NA	NA	NA	7
	2007	6	NA	NA	NA	NA	NA	NA	6
	2008	10	350	350	350	350	350	350	9
	2009	8	20	103	185	185	268	350	6
	2010	8	20	210	400	307	450	500	5
	2011	5	20	20	20	20	20	20	4
	2012	6	NA	NA	NA	NA	NA	NA	6
	2013	7	20	20	20	20	20	20	6
	2014	8	20	20	20	20	20	20	7
	2015	3	NA	NA	NA	NA	NA	NA	3
Trade herds	2006	0	NA	NA	NA	NA	NA	NA	0
	2007	1	10	10	10	10	10	10	0
	2008	1	1,010	1,010	1,010	1,010	1,010	1,010	0
	2009	7	14	58	880	909	1,400	2,555	0
	2010	20	2	219	890	998	1,765	2,555	0
	2011	17	1	350	940	1,294	2,200	4,501	0
	2012	17	2	350	940	1,107	1,720	2,900	0
	2013	16	2	513	971	1,154	1,840	2,900	0
	2014	15	2	600	1,002	1,230	1,960	2,900	0
	2015	16	2	100	983	1,090	2,000	2,900	2

		Number of active holdings	Minimum	1st quantile	Median	Mean	3rd quantile	Maximum	Not available
Pig shows	2006	9	1	1	1	1	1	1	8
	2007	8	1	6	10	7	11	11	5
	2008	8	1	1	1	1	1	1	6
	2009	6	1	4	6	6	9	11	4
	2010	6	1	4	6	6	9	11	4
	2011	6	1	4	6	6	9	11	4
	2012	9	1	4	6	6	9	11	7
	2013	9	1	4	6	6	9	11	7
	2014	6	1	4	6	6	9	11	4
	2015	7	1	4	6	6	9	11	5
Livestock auctions	2006	1	NA	NA	NA	NA	NA	NA	1
	2007	1	NA	NA	NA	NA	NA	NA	1
	2008	0	NA	NA	NA	NA	NA	NA	0
	2009	0	NA	NA	NA	NA	NA	NA	0
	2010	0	NA	NA	NA	NA	NA	NA	0
	2011	0	NA	NA	NA	NA	NA	NA	0
	2012	0	NA	NA	NA	NA	NA	NA	0
	2013	0	NA	NA	NA	NA	NA	NA	0
	2014	0	NA	NA	NA	NA	NA	NA	0
	2015	0	NA	NA	NA	NA	NA	NA	0
Collection points (CP)	2006	7	NA	NA	NA	NA	NA	NA	7
	2007	11	1	136	270	374	560	850	8
	2008	15	1	1	1	67	101	200	12
	2009	16	1	1	3	341	200	1,500	11
	2010	17	1	51	200	484	800	1,500	11
	2011	17	1	200	500	640	1,000	1,500	12
	2012	20	1	51	350	534	875	1,500	14
	2013	21	1	51	350	534	875	1,500	15
	2014	24	1	51	350	534	875	1,500	18
	2015	25	1	101	500	715	1,250	1,800	18

		Number of active holdings	Minimum	1st quantile	Median	Mean	3rd quantile	Maximum	Not available
Slaughter animal markets	2006	1	NA	NA	NA	NA	NA	NA	1
	2007	2	NA	NA	NA	NA	NA	NA	2
	2008	2	NA	NA	NA	NA	NA	NA	2
	2009	0	NA	NA	NA	NA	NA	NA	0
	2010	0	NA	NA	NA	NA	NA	NA	0
	2011	0	NA	NA	NA	NA	NA	NA	0
	2012	0	NA	NA	NA	NA	NA	NA	0
	2013	1	NA	NA	NA	NA	NA	NA	1
	2014	1	NA	NA	NA	NA	NA	NA	1
	2015	1	NA	NA	NA	NA	NA	NA	1

Table 7. Descriptive summaries of holding sizes for miscellaneous sites.

		Number of active holdings	Minimum	1st quantile	Median	Mean	3rd quantile	Maximum	Not available
Zoos	2006	0	NA	NA	NA	NA	NA	NA	0
	2007	0	NA	NA	NA	NA	NA	NA	0
	2008	0	NA	NA	NA	NA	NA	NA	0
	2009	0	NA	NA	NA	NA	NA	NA	0
	2010	0	NA	NA	NA	NA	NA	NA	0
	2011	1	20	20	20	20	20	20	0
	2012	1	NA	NA	NA	NA	NA	NA	1
	2013	4	20	20	20	20	20	20	3
	2014	4	NA	NA	NA	NA	NA	NA	4
	2015	4	3	4	5	5	6	6	0
Experimental facilities	2006	0	NA	NA	NA	NA	NA	NA	0
	2007	0	NA	NA	NA	NA	NA	NA	0
	2008	1	400	400	400	400	400	400	0
	2009	1	100	100	100	100	100	100	0
	2010	3	3	52	102	102	151	200	1
	2011	3	4	12	20	141	210	400	0
	2012	2	3	7	12	12	16	20	0
	2013	1	20	20	20	20	20	20	0
	2014	2	20	20	20	20	20	20	1
	2015	5	1	1	30	116	100	450	0

Table 8. Descriptive summaries of holding sizes for end of production sites.

		Number of active holdings	Minimum	1st quantile	Median	Mean	3rd quantile	Maximum	Not available
Slaughterhouses	2006	100	1	3	4	38	57	110	97
	2007	97	1	4	1,800	1,854	1,975	5,491	92
	2008	89	1	45	870	885	1,710	1,800	85
	2009	83	1	1	1	561	841	1,680	80
	2010	80	1	1	1	1	1	1	78
	2011	78	1	1	1	1	1	1	76
	2012	74	1	1	1	1	1	1	72
	2013	75	1	1	1	1	1	1	73
	2014	77	1	1	1	1	1	1	75
	2015	76	1	1	1	1	1	1	75
Export isolation facilities	2006	0	NA	NA	NA	NA	NA	NA	0
	2007	0	NA	NA	NA	NA	NA	NA	0
	2008	0	NA	NA	NA	NA	NA	NA	0
	2009	0	NA	NA	NA	NA	NA	NA	0
	2010	0	NA	NA	NA	NA	NA	NA	0
	2011	3	150	165	180	260	315	450	0
	2012	3	27	104	180	219	315	450	0
	2013	3	27	104	180	219	315	450	0
	2014	3	27	104	180	219	315	450	0
	2015	3	27	104	180	219	315	450	0

		Number of active holdings	Minimum	1st quantile	Median	Mean	3rd quantile	Maximum	Not available
CPs for dead animals	2006	2	NA	NA	NA	NA	NA	NA	2
	2007	4	6	11	16	16	20	25	2
	2008	2	NA	NA	NA	NA	NA	NA	2
	2009	2	240	280	320	320	360	400	0
	2010	2	25	25	25	25	25	25	1
	2011	2	1,620	1,620	1,620	1,620	1,620	1,620	1
	2012	7	10	83	155	155	228	300	5
	2013	18	10	83	155	155	228	300	16
	2014	35	10	83	155	155	228	300	33
	2015	121	300	300	300	300	300	300	120
Cooling stations	2006	1	NA	NA	NA	NA	NA	NA	1
	2007	1	NA	NA	NA	NA	NA	NA	1
	2008	1	NA	NA	NA	NA	NA	NA	1
	2009	1	180	180	180	180	180	180	0
	2010	1	NA	NA	NA	NA	NA	NA	1
	2011	1	420	420	420	420	420	420	0
	2012	9	NA	NA	NA	NA	NA	NA	9
	2013	30	NA	NA	NA	NA	NA	NA	30
	2014	33	NA	NA	NA	NA	NA	NA	33
	2015	51	NA	NA	NA	NA	NA	NA	51
Rendering plants	2006	1	NA	NA	NA	NA	NA	NA	1
	2007	1	NA	NA	NA	NA	NA	NA	1
	2008	1	NA	NA	NA	NA	NA	NA	1
	2009	1	NA	NA	NA	NA	NA	NA	1
	2010	1	NA	NA	NA	NA	NA	NA	1
	2011	1	NA	NA	NA	NA	NA	NA	1
	2012	1	NA	NA	NA	NA	NA	NA	1
	2013	1	NA	NA	NA	NA	NA	NA	1
	2014	1	NA	NA	NA	NA	NA	NA	1
	2015	1	NA	NA	NA	NA	NA	NA	1

9.1.2. S2 File: Number of pigs per movment

Table 1. Batch sizes. Descriptive statistics of the development of the number of pigs per movment (batch size) of registered pig movements between 1 Jan 2006 and 31 Dec 2015 in Denmark. The column "not available" covers both the number of pig movements without batch sizes and with batch size equal to 0.

	Minimum	1st Quantile	Median	Mean	3rd Quantile	Maximum	Not available
2006	1	2	8	49	60	9,780	45,092
2007	1	2	9	53	65	9,000	40,811
2008	1	3	10	61	76	7,187	38,705
2009	1	2	9	67	82	7,035	37,003
2010	1	3	10	75	93	7,893	35,843
2011	1	3	10	79	100	9,381	37,481
2012	1	3	11	83	105	6,918	37,408
2013	1	3	11	85	108	7,071	37,769
2014	1	3	11	90	115	8,721	40,636
2015	1	3	11	96	125	7,588	43,221

Table 2. Median batch size. Median batch size by holding type of sending holding of registered pig movements between 1 Jan 2006 and 31 Dec 2015 in Denmark.

	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Breeding sites										
Breeding and multiplier herds	10	10	11	11	14	15	15	15	16	19
Quarantine stations	11	10	12	10	8	11	11	11	12	10
Boar stations	3	3	2	2	2	2	2	2	2	2
Production sites										
Production herds	8	9	10	9	10	10	11	11	11	11
Weaner herds	60	55	57	29	16	15	15	15	19	20
Free-ranging pig herds	7	7	6	5	5	5	5	5	5	5
Organic pig herds	5	7	8	8	9	8	9	9	7	8
Hobby sites										
Hobby herds	3	3	3	3	3	3	4	5	5	3
Pets	3	4	3	147	7	4		4	4	4
Wild boar herds		2		1	2		2	10	6	3
Organic wild boar herds										2
Transit sites										
Traders	200	235	52	35	11	22	13	13	38	71
Trade herds		6	20	8	11	10	10	11	13	10
Pig shows	3	3	3	11	15	14	6	5	15	8
Livestock auctions	11	10								
Collection points (CP)	4	4	4	4	6	8	12	13	10	10
Slaughter animal markets	1	5	5					108		7
Miscellaneous										
Zoos						2	1	2	2	2
Experimental facilities					2		3			8

	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
End of production sites										
Slaughterhouses	3	3	2	4	4	3	2	2	2	2
Export isolation facilities						2	1	2	2	2
CPs for dead animals	3	3	302	4	7	3	2	2	3	3
Cooling stations	2	286	4	1	3	4	4	9	11	10
Rendering plants										

Table 3. Median batch size by holding type of receiving holding of registered pig movements between 1 Jan 2006 and 31 Dec 2015 in Denmark.

	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Breeding sites										
Breeding and multiplier herds	103	120	120	120	126	147	153	165	169	170
Quarantine stations	7	10	5	12	5	5	4	4	5	4
Boar stations	14	17	16	15	11	13	12	16	19	18
Production sites										
Production herds	141	151	180	202	220	240	250	251	255	263
Weaner herds	295	298	325	337	303	401	400	398	396	406
Free-ranging pig herds	119	42	46	115	25	25	20	7	32	41
Organic pig herds	50	85	127	150	188	186	205	228	149	175
Hobby sites										
Hobby herds	5	106	75	166	260	6	5	24	6	4
Pets	30	10		324	171	36	2	2	630	2
Wild boar herds		8	2			2	7	58	2	3
Organic wild boar herds										
Transit sites										
Traders	44	10	30	28	174	270	250	60	78	53
Trade herds				216	81	14	12	12	12	154
Pig shows	4	4	3	6	2	2	4	2	2	2
Livestock auctions	180	15								
Collection points (CP)	11	15	18	70	73	80	109	151	254	285
Slaughter animal markets	249	116	15						661	634
Miscellaneous										
Zoos							1	7	3	3
Experimental facilities			24	22	16	14	14	16	17	10

	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
End of production sites										
Slaughterhouses	35	39	44	45	48	52	54	53	58	67
Export isolation facilities							312	65	44	91
CPs for dead animals			325	118	2	220	7	2	4	3
Cooling stations		331					2	1	2	3
Rendering plants	2	2	2	2	2	2	2	2	2	2

Table 4. Average number of pig movements per holding type of sending holding per year between 1 Jan 2006 and 31 Dec 2015 in Denmark.

	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Breeding sites										
Breeding and multiplier herds	157	167	166	163	148	144	135	137	142	151
Quarantine stations	23	20	17	20	19	20	20	23	27	22
Boar stations	40	43	45	48	47	47	46	44	46	51
Production sites										
Production herds	75	80	79	82	87	90	87	88	89	90
Weaner herds	78	81	89	104	108	106	104	102	106	107
Free-ranging pig herds	37	33	33	24	20	19	18	16	18	15
Organic pig herds	32	36	40	36	40	46	49	47	46	52
Hobby sites										
Hobby herds	4	10	8	7	4	4	4	5	7	3
Pets	1	1	1	19	8	1		9	1	2
Wild boar herds		1		1	1		6	13	1	3
Organic wild boar herds										3
Transit sites										
Traders	5	9	3	16	20	15	8	4	10	10
Trade herds		1	75	70	61	68	72	83	102	66
Pig shows	1	2	1	1	1	1	2	2	1	1
Livestock auctions	215	244								
Collection points (CP)	82	71	93	73	94	67	69	59	57	63
Slaughter animal markets	41	77	112					3		9
Miscellaneous										
Zoos						1	1	2	1	2
Experimental facilities					1		18			28

	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
End of production sites										
Slaughterhouses	165	87	76	166	180	96	74	80	96	90
Export isolation facilities						3	3	11	12	5
CPs for dead animals	51	1	6	5	3	57	51	23	32	54
Cooling stations	79	41	1	1	4	1	34	7	26	35
Rendering plants										

Table 5. Average number of pig movements per holding type of receiving holding per year between 1 Jan 2006 and 31 Dec 2015 in Denmark.

	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Breeding sites										
Breeding and multiplier herds	13	15	18	19	20	21	20	21	22	21
Quarantine stations	39	52	19	28	20	24	25	26	29	31
Boar stations	21	19	20	22	22	20	20	17	15	18
Production sites										
Production herds	12	14	14	15	16	15	16	16	17	17
Weaner herds	24	29	29	25	23	22	21	21	23	24
Free-ranging pig herds	6	6	6	6	4	3	2	3	6	2
Organic pig herds	3	3	4	5	4	4	5	5	8	7
Hobby sites										
Hobby herds	1	4	9	4	9	2	3	5	6	1
Pets	2	1		12	4	2	1	1	4	1
Wild boar herds		1	1			2	1	4	1	2
Organic wild boar herds										
Transit sites										
Traders	15	6	5	6	25	93	24	5	8	52
Trade herds				14	21	41	62	81	78	22
Pig shows	1	2	1	2	2	2	2	3	3	2
Livestock auctions	539	585								
Collection points (CP)	2,804	2,467	1,892	1,445	1,319	1,451	1,183	1,149	834	836
Slaughter animal markets	217	512	1,299						1	2
Miscellaneous										
Zoos							1	1	2	2
Experimental facilities			9	16	24	23	25	52	26	51

	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
End of production sites										
Slaughterhouses	4,272	4,095	3,858	3,682	3,665	3,752	3,498	3,419	3,074	2,797
Export isolation facilities							3	2	12	4
CPs for dead animals			8	1	1	5	43	48	27	55
Cooling stations		40					23	38	64	86
Rendering plants	401,386	405,473	370,127	350,848	340,541	333,422	302,656	295,889	297,428	295,306

9.1.3. S3 File: In- and out-loyalty per holding type

Figure 1. In-loyalty. In-loyalty for each pair of consecutive years for the whole network of pig movements from 1 Jan 2006 to 31 Dec 2015 in Denmark.

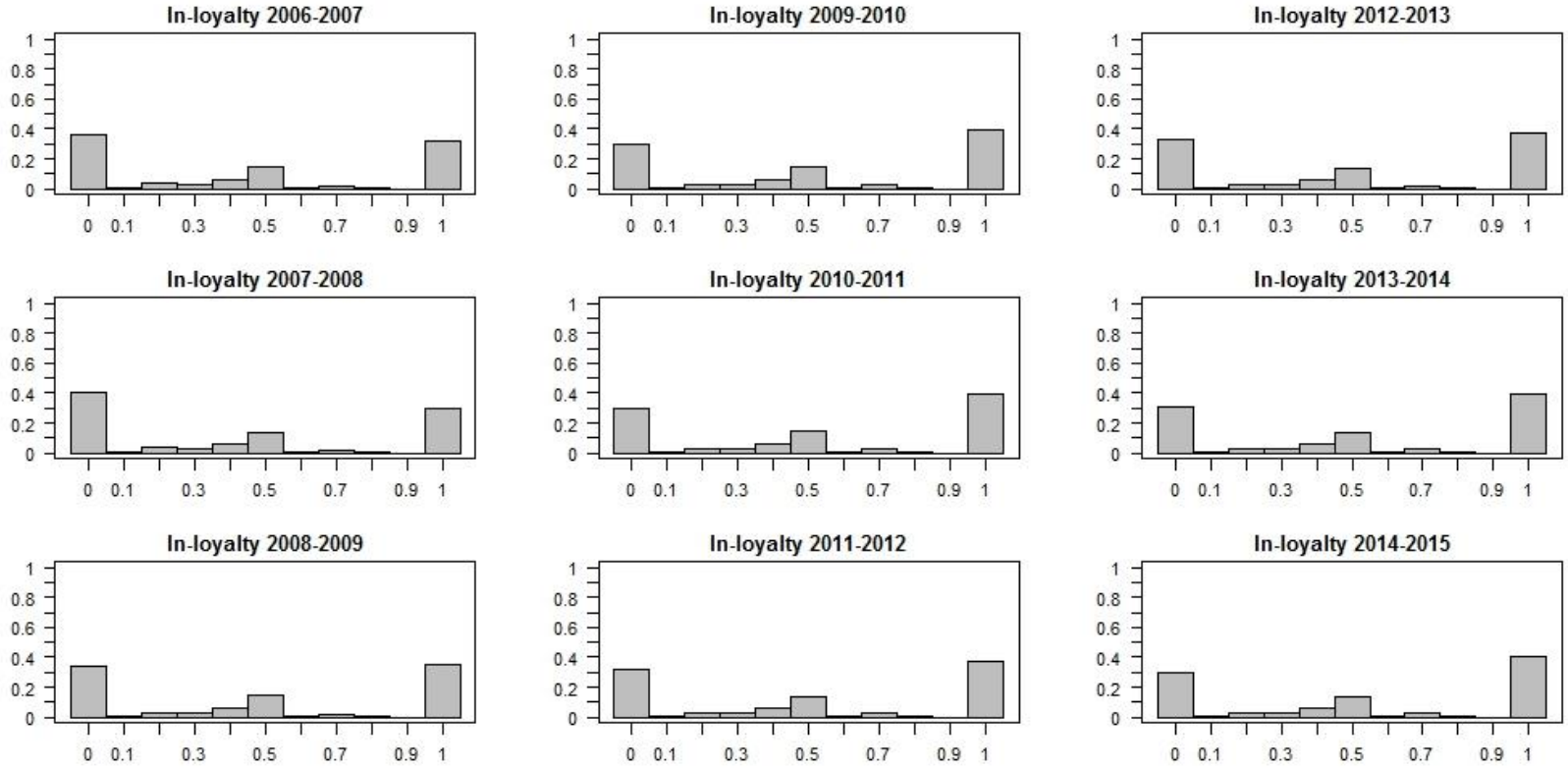


Figure 2. Out-loyalty. Out-loyalty for each pair of consecutive years for the whole network of pig movements from 1 Jan 2006 to 31 Dec 2015 in Denmark.

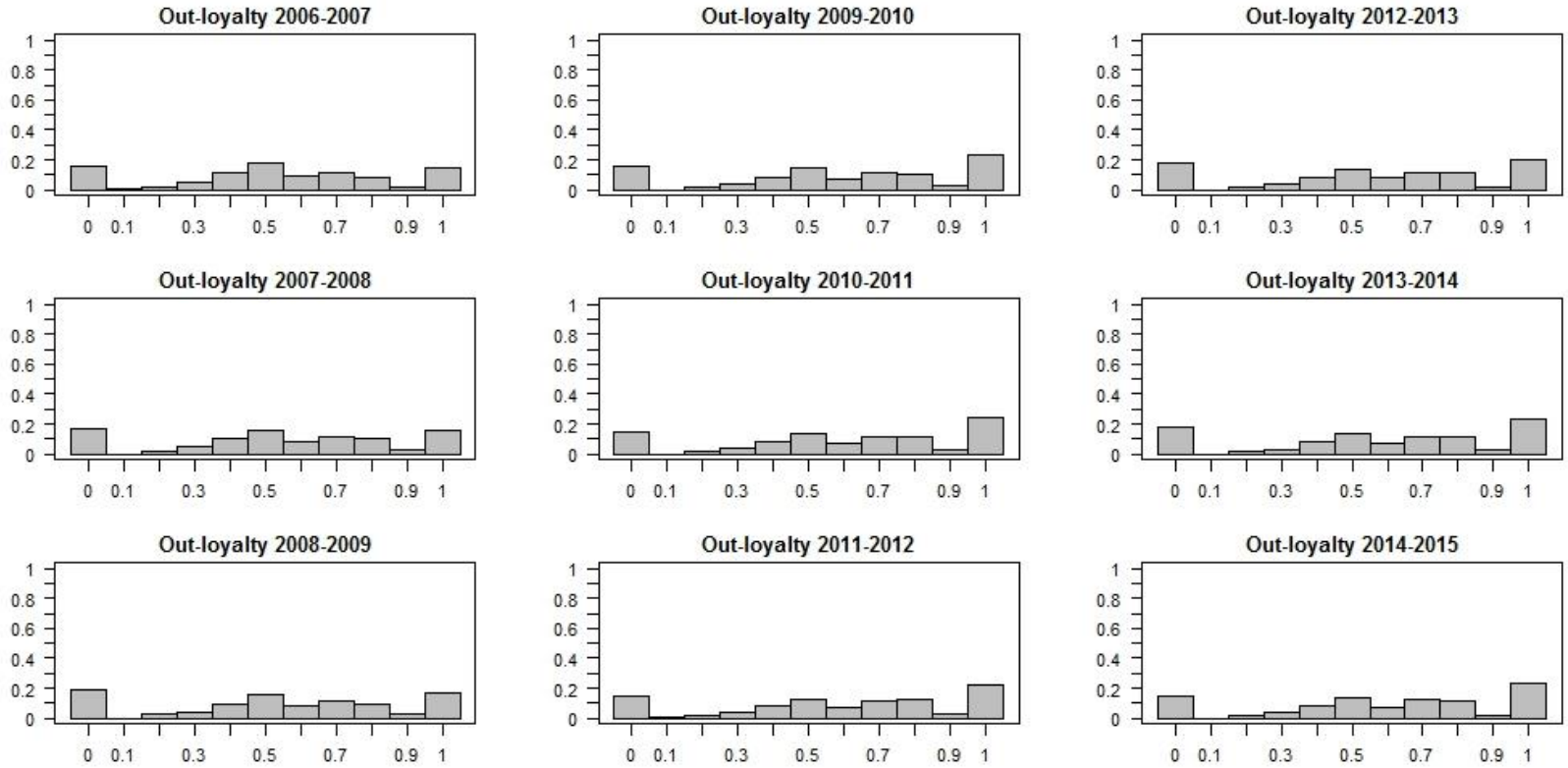


Figure 3. In- and out-loyalty for breeding sites.

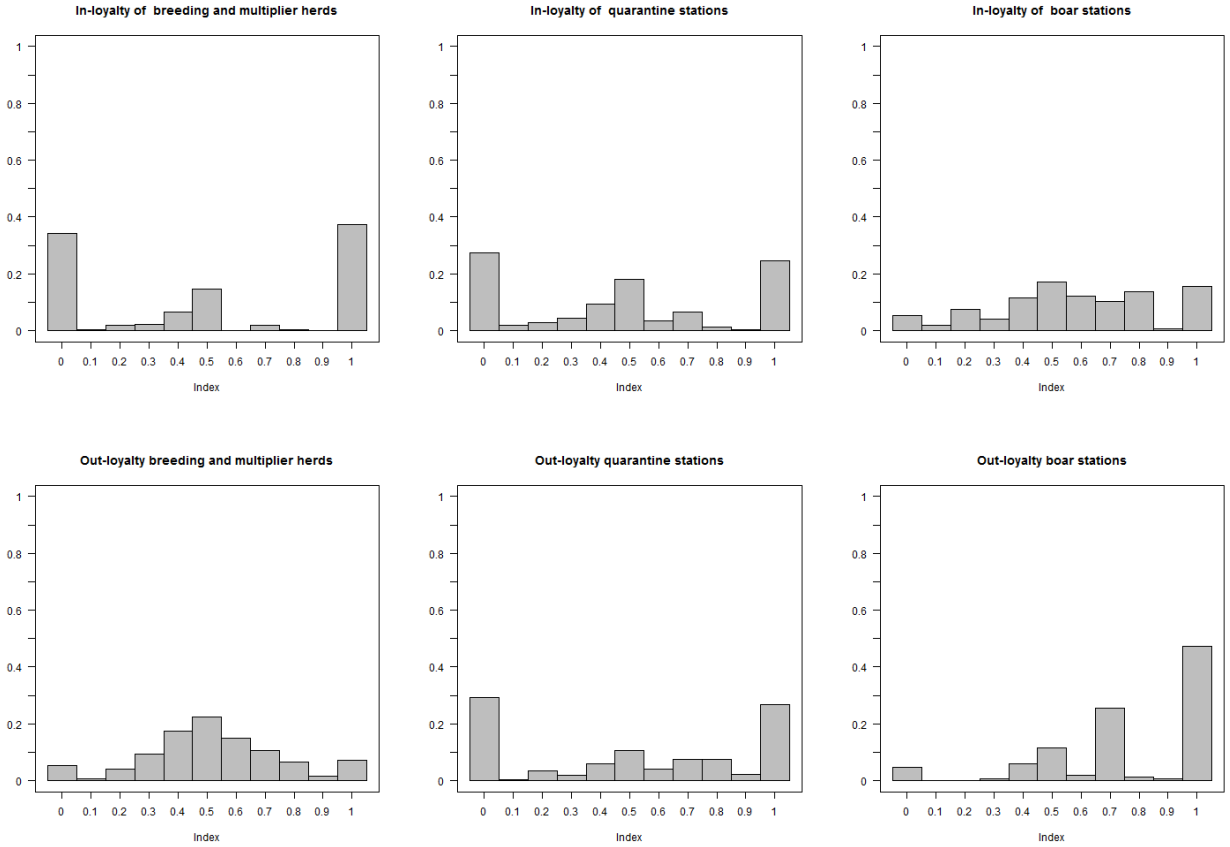


Figure 4. In- and out-loyalty for production sites.

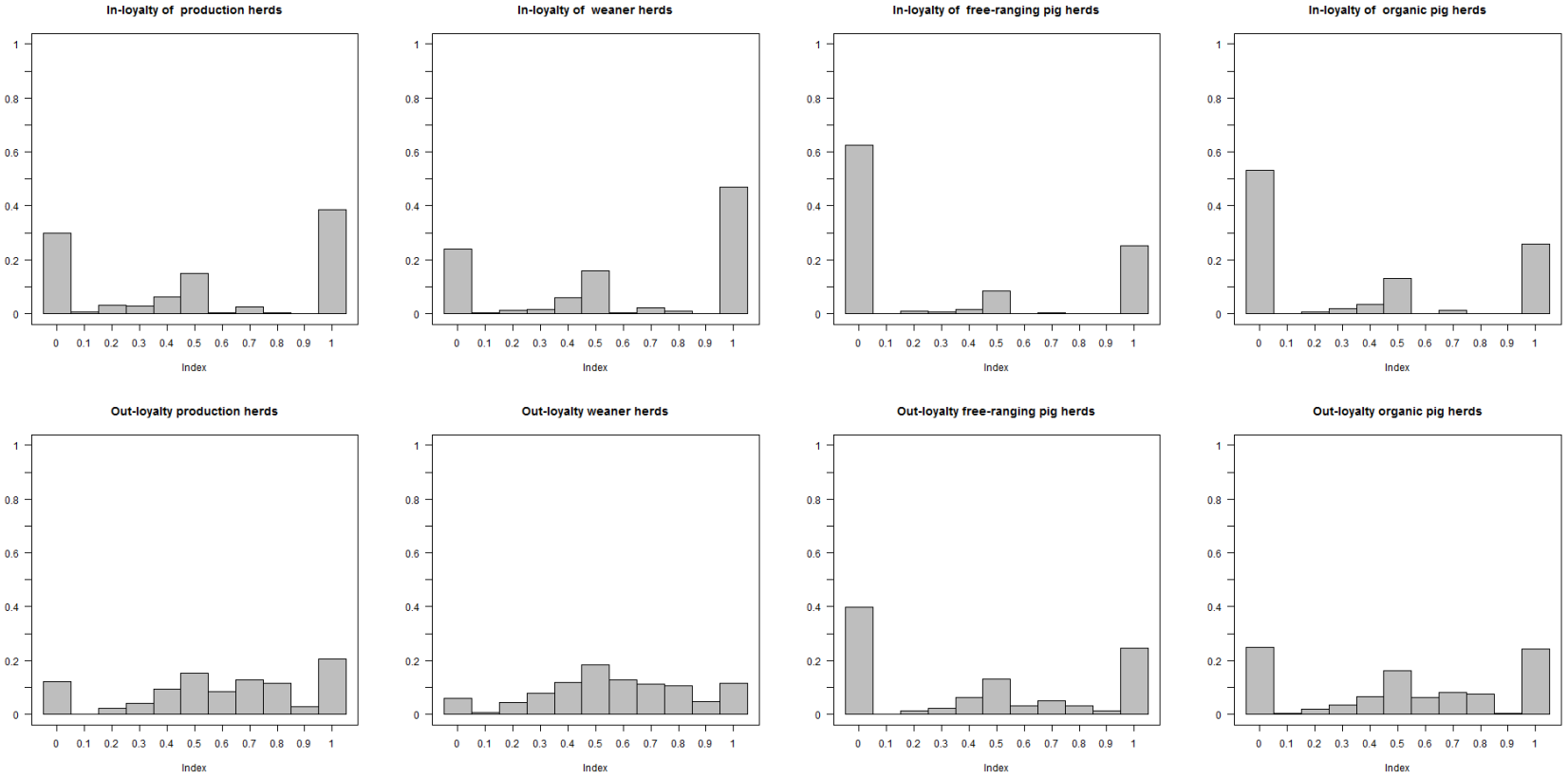


Figure 5. In- and out-loyalty for hobby sites.

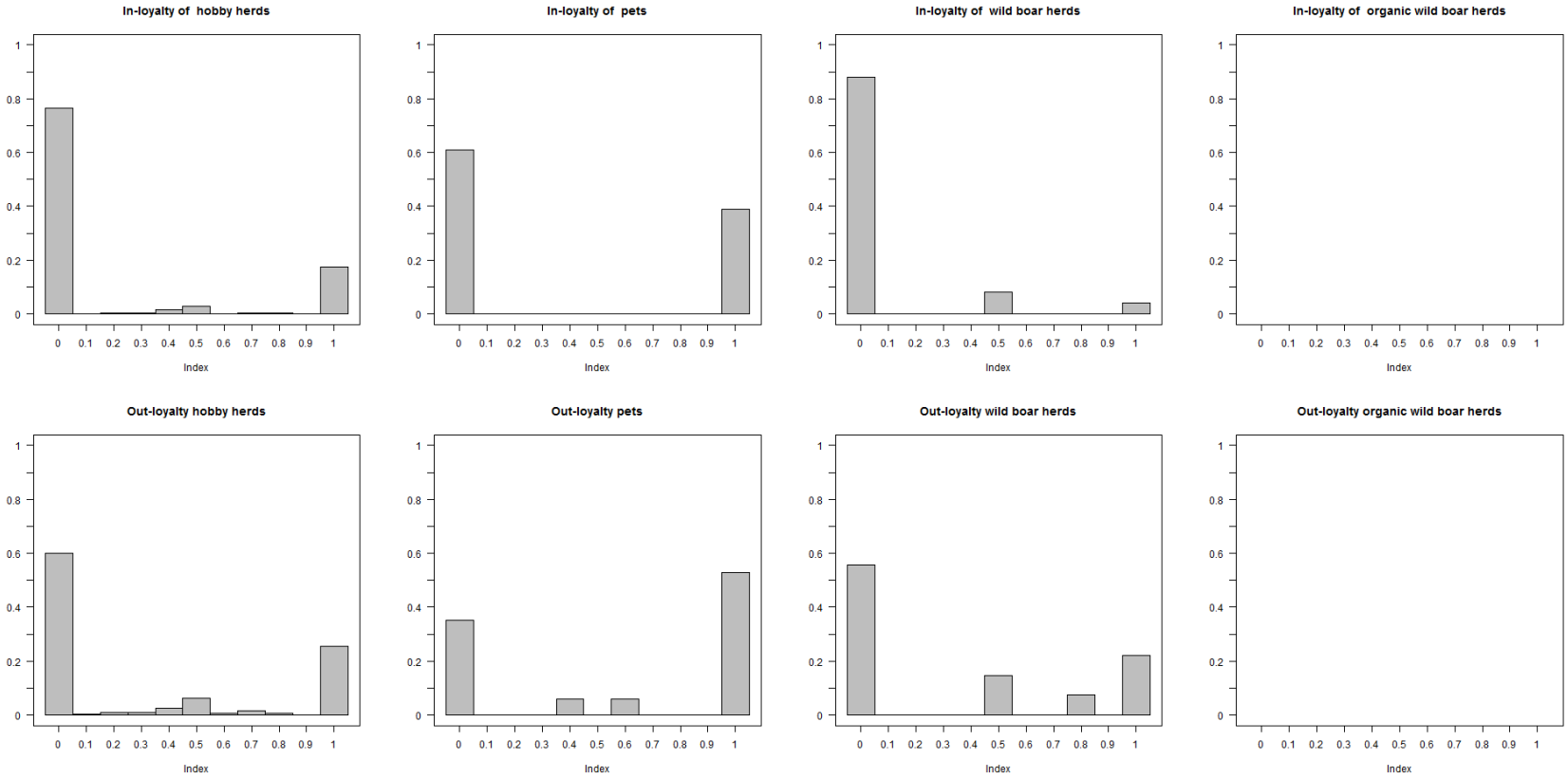


Figure 6. In- and out-loyalty for transit sites.

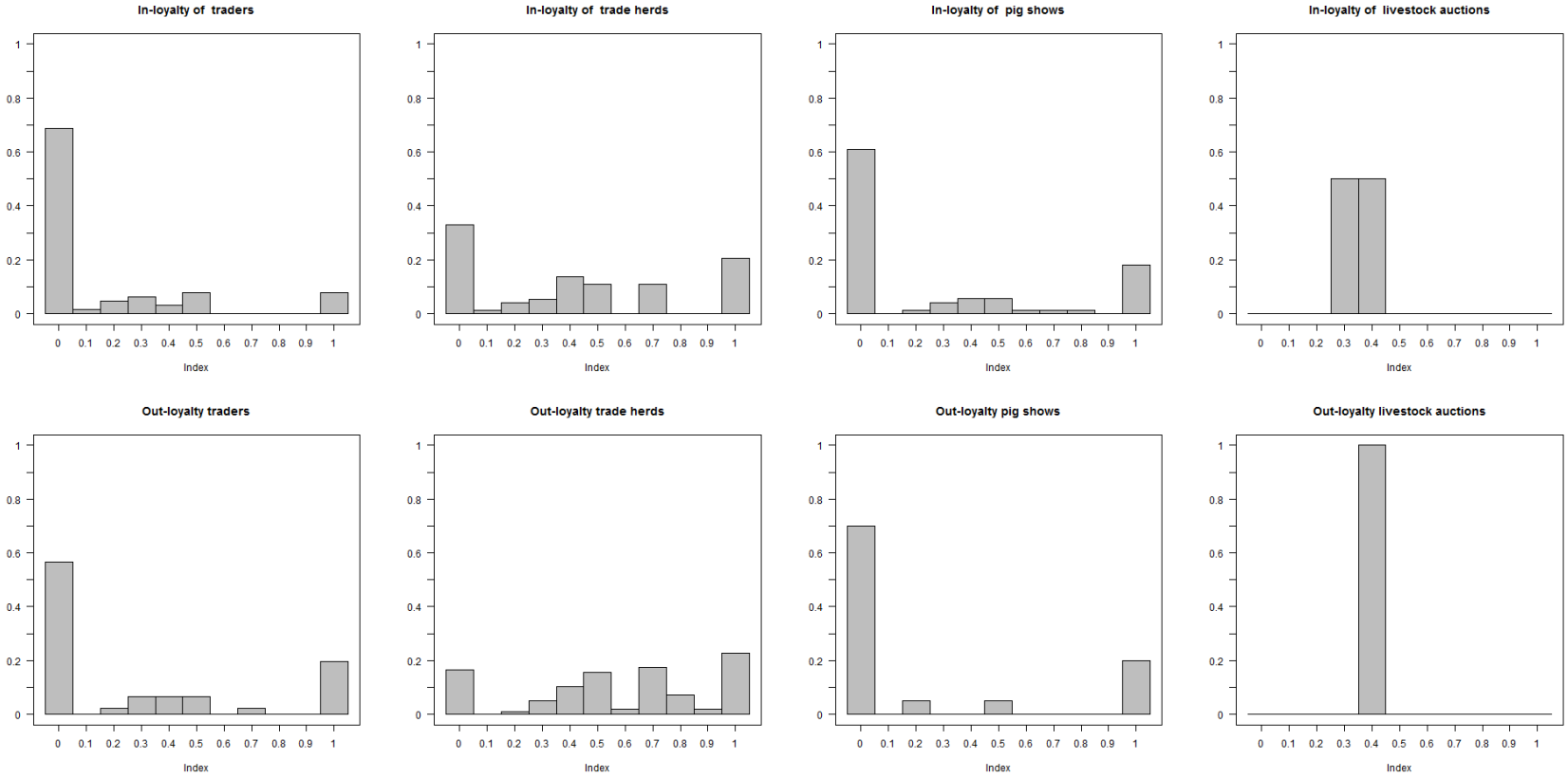


Figure 6 (continued). In- and out-loyalty for transit sites.

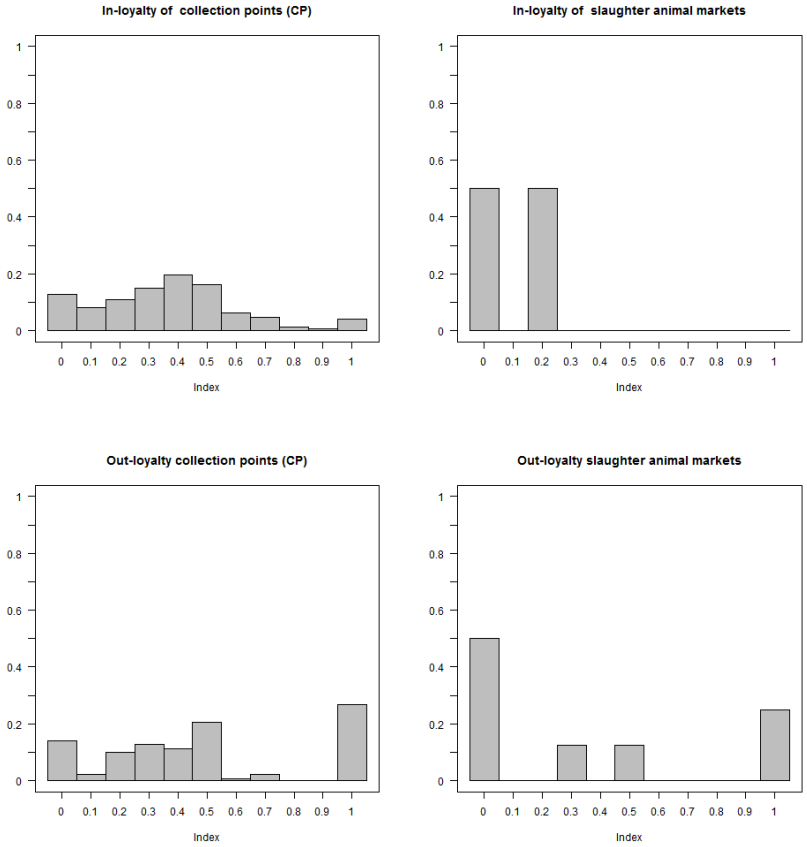


Figure 7. In- and out-loyalty for miscellaneous sites.

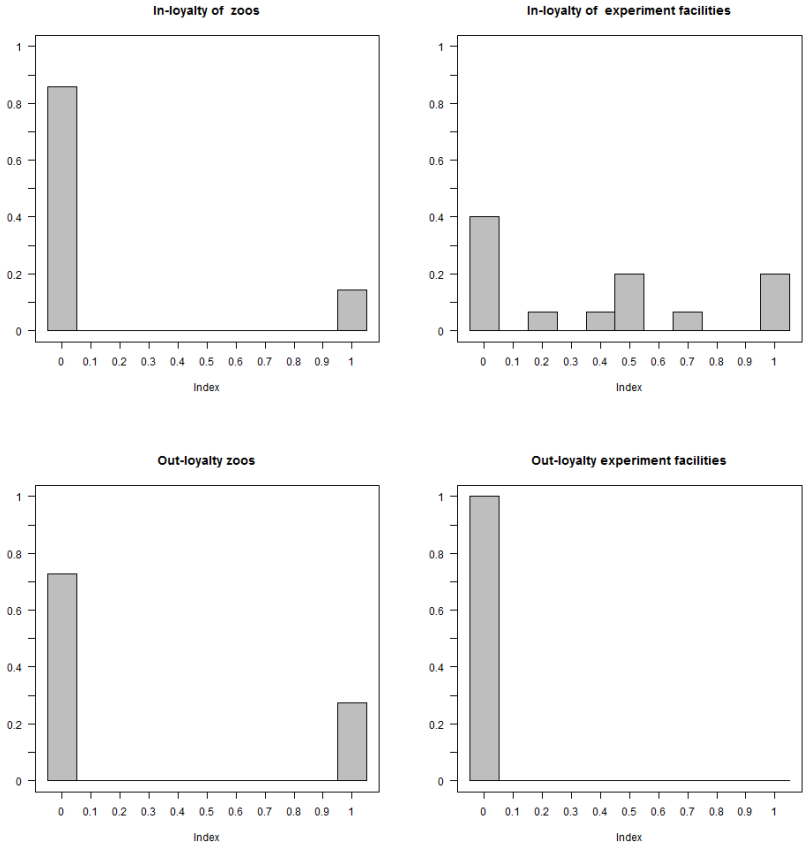


Figure 8. In- and out-loyalty for end of production sites.

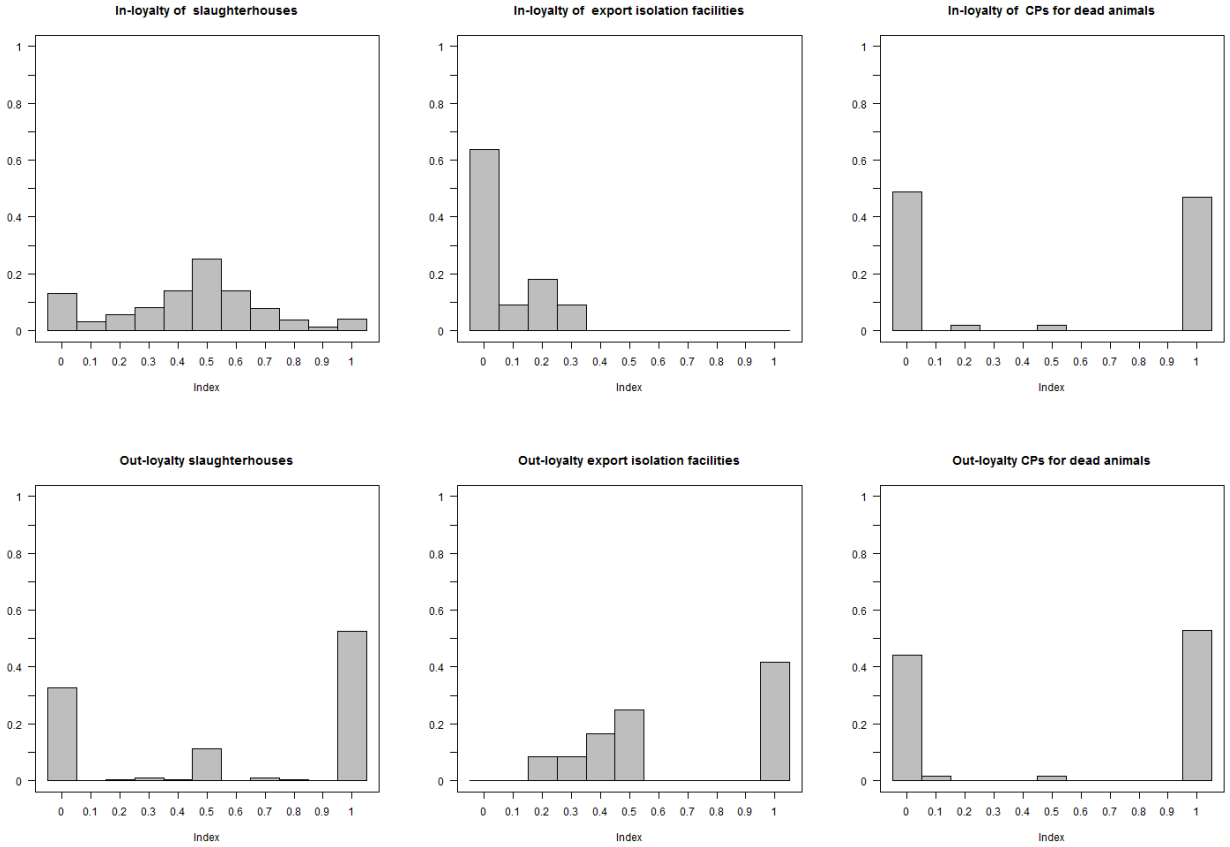


Figure 8 (continued). In- and out-loyalty for end of production sites.

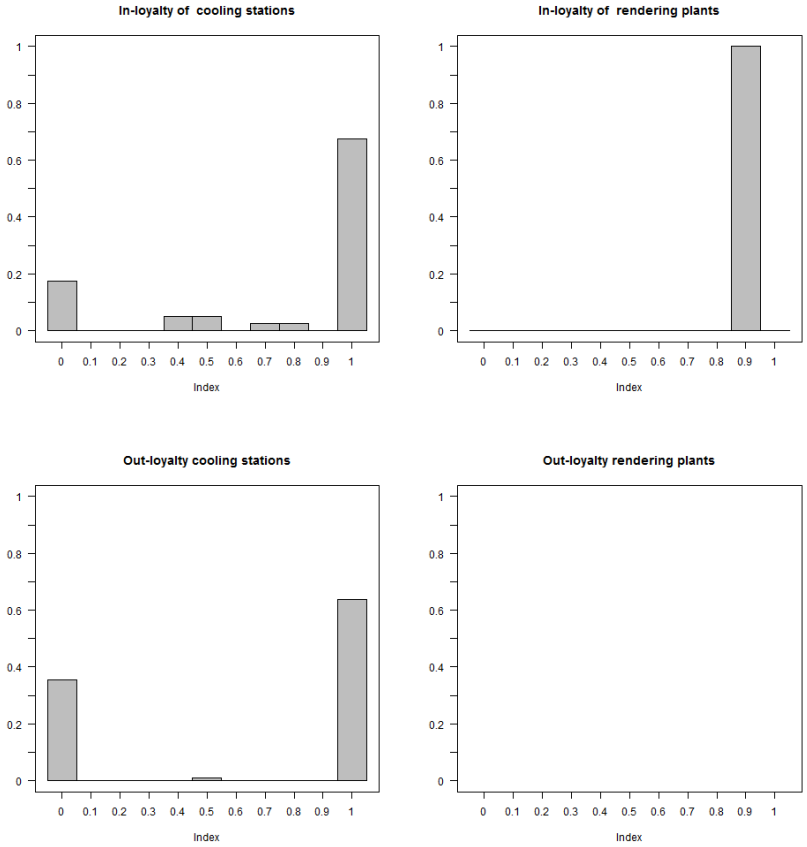


Table 1. Summary of in-loyalty per holding type. Levels for in-loyalty: (1) low – mean < 0.45, (2) intermediate – 0.45 ≤ mean ≤ 0.55 and (3) high – mean > 0.55

	Minimum	1st quantile	Median	Mean	3rd quantile	Maximum	Level
Breeding sites							
Breeding and multiplier herds	0,00	0,00	0,50	0,50	1,00	1,00	intermediate
Quarantine stations	0,00	0,00	0,49	0,46	0,78	1,00	intermediate
Boar stations	0,00	0,38	0,58	0,55	0,75	1,00	intermediate
Production sites							
Production herds	0,00	0,00	0,50	0,52	1,00	1,00	intermediate
Weaner herds	0,00	0,17	0,67	0,60	1,00	1,00	high
Free-ranging pig herds	0,00	0,00	0,00	0,31	1,00	1,00	low
Organic pig herds	0,00	0,00	0,00	0,35	1,00	1,00	low
Hobby sites							
Hobby herds	0,00	0,00	0,00	0,20	0,00	1,00	low
Pets	0,00	0,00	0,00	0,39	1,00	1,00	low
Wild boar herds	0,00	0,00	0,00	0,08	0,00	1,00	low
Organic wild boar herds	NA	NA	NA	NA	NA	NA	not available
Transit sites							
Traders	0,00	0,00	0,00	0,15	0,20	1,00	low
Trade herds	0,00	0,00	0,35	0,40	0,67	1,00	low
Pig shows	0,00	0,00	0,00	0,27	0,50	1,00	low
Livestock auctions	0,25	0,27	0,29	0,29	0,30	0,32	low
Collection points (CP)	0,00	0,13	0,33	0,32	0,44	1,00	low
Slaughter animal markets	0,00	0,00	0,05	0,06	0,12	0,14	low
Miscellaneous							
Zoos	0,00	0,00	0,00	0,14	0,00	1,00	low
Experimental facilities	0,00	0,00	0,33	0,38	0,58	1,00	low

	Minimum	1st quantile	Median	Mean	3rd quantile	Maximum	Level
End of production sites							
Slaughterhouses	0,00	0,27	0,43	0,40	0,54	1,00	low
Export isolation facilities	0,00	0,00	0,00	0,06	0,11	0,29	low
CPs for dead animals	0,00	0,00	0,18	0,48	1,00	1,00	intermediate
Cooling stations	0,00	0,50	1,00	0,75	1,00	1,00	high
Rendering plants	0,81	0,85	0,86	0,86	0,87	0,89	high

Table 2. Summary of out-loyalty per holding type. Levels for out-loyalty: (1) low – mean < 0.45, (2) intermediate – 0.45 ≤ mean ≤ 0.55 and (3) high – mean > 0.55

	Minimum	1st quantile	Median	Mean	3rd quantile	Maximum	
Breeding sites							
Breeding and multiplier herds	0,00	0,33	0,49	0,49	0,62	1,00	intermediate
Quarantine stations	0,00	0,00	0,50	0,50	1,00	1,00	intermediate
Boar stations	0,00	0,63	0,67	0,75	1,00	1,00	high
Production sites							
Production herds	0,00	0,38	0,60	0,58	0,80	1,00	high
Weaner herds	0,00	0,36	0,54	0,54	0,71	1,00	intermediate
Free-ranging pig herds	0,00	0,00	0,43	0,43	0,83	1,00	low
Organic pig herds	0,00	0,08	0,50	0,51	0,80	1,00	intermediate
Hobby sites							
Hobby herds	0,00	0,00	0,00	0,32	1,00	1,00	low
Pets	0,00	0,00	1,00	0,58	1,00	1,00	high
Wild boar herds	0,00	0,00	0,00	0,35	0,75	1,00	low
Organic wild boar herds	NA	NA	NA	NA	NA	NA	not available
Transit sites							
Traders	0,00	0,00	0,00	0,29	0,50	1,00	low
Trade herds	0,00	0,35	0,60	0,56	0,80	1,00	high
Pig shows	0,00	0,00	0,00	0,24	0,28	1,00	low
Livestock auctions	0,36	0,36	0,37	0,37	0,37	0,38	low
Collection points (CP)	0,00	0,20	0,41	0,47	1,00	1,00	intermediate
Slaughter animal markets	0,00	0,00	0,13	0,34	0,63	1,00	low
Miscellaneous							
Zoos	0,00	0,00	0,00	0,27	0,50	1,00	low
Experimental facilities	0,00	0,00	0,00	0,00	0,00	0,00	low

	Minimum	1st quantile	Median	Mean	3rd quantile	Maximum	
End of production sites							
Slaughterhouses	0,00	0,00	1,00	0,60	1,00	1,00	high
Export isolation facilities	0,20	0,38	0,46	0,63	1,00	1,00	high
CPs for dead animals	0,00	0,00	1,00	0,54	1,00	1,00	intermediate
Cooling stations	0,00	0,00	1,00	0,64	1,00	1,00	high
Rendering plants	NA	NA	NA	NA	NA	NA	not available

9.1.4. S4 File: Ingoing- and outgoing contact chains

Figure 1. Contact chains. Size of (a) in-going and (b) out-going contact chain for the whole pig movement network in Denmark from 1 Jan 2006 to 31 Dec 2015.

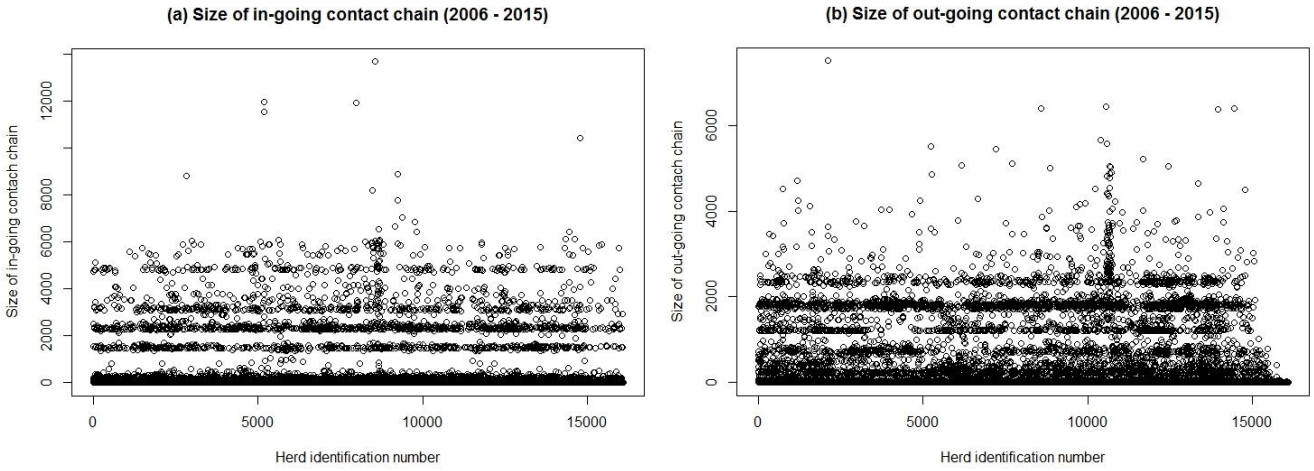


Figure 2. In-going and out-going contact chains for breeding sites.

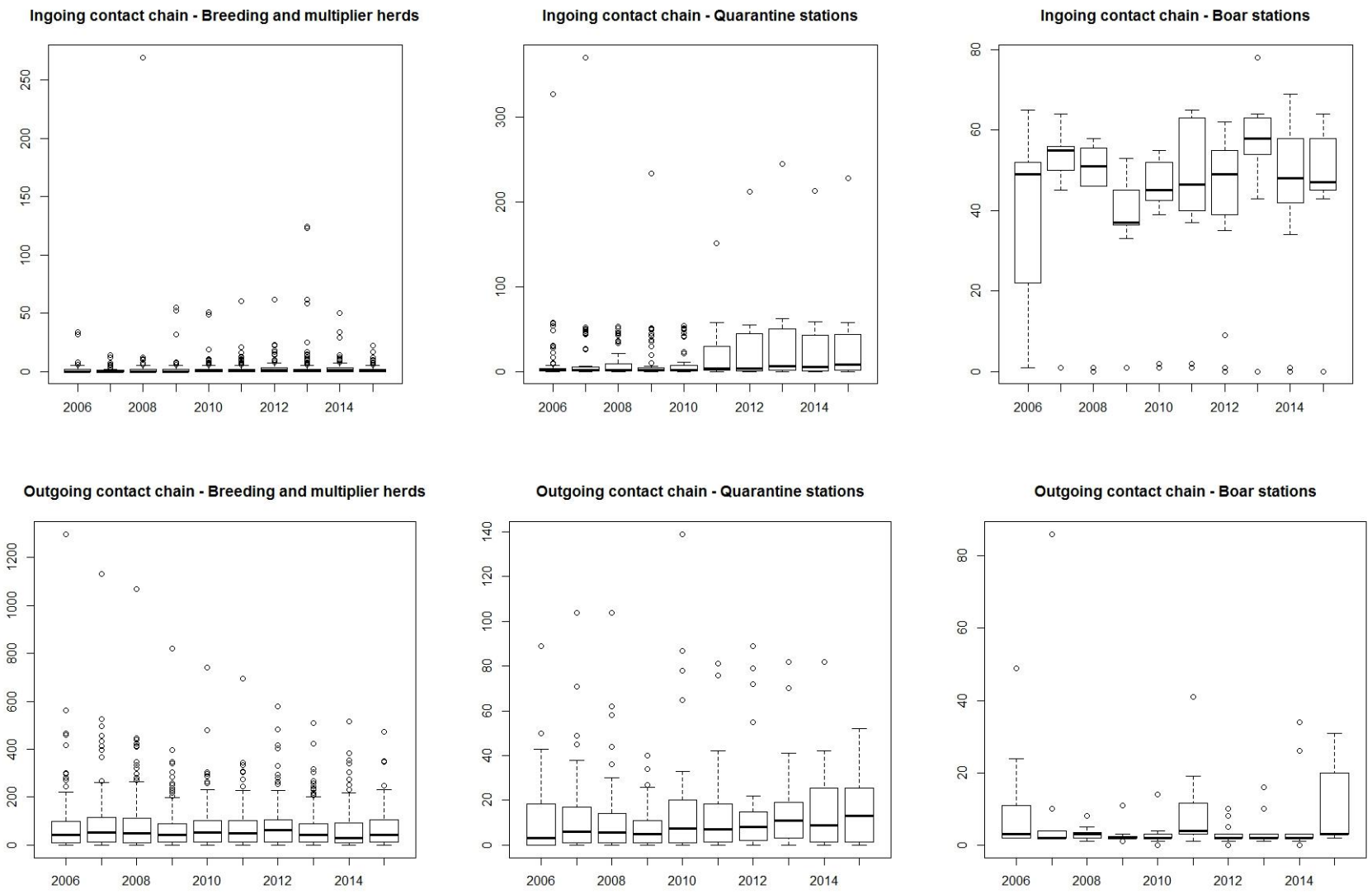


Figure 3. In-going and out-going contact chains for production sites.

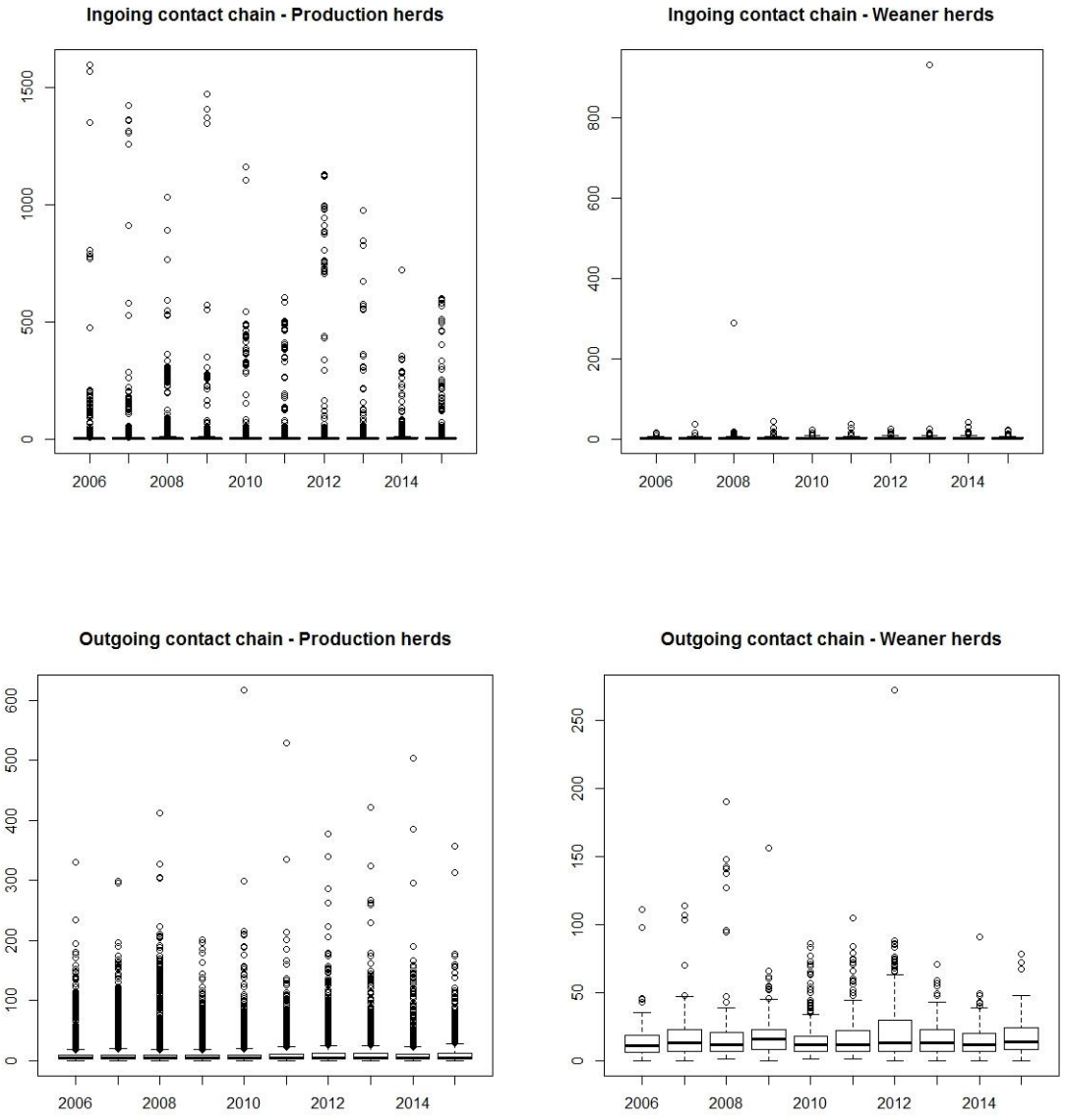


Figure 3 (continued). In-going and out-going contact chains for production sites.

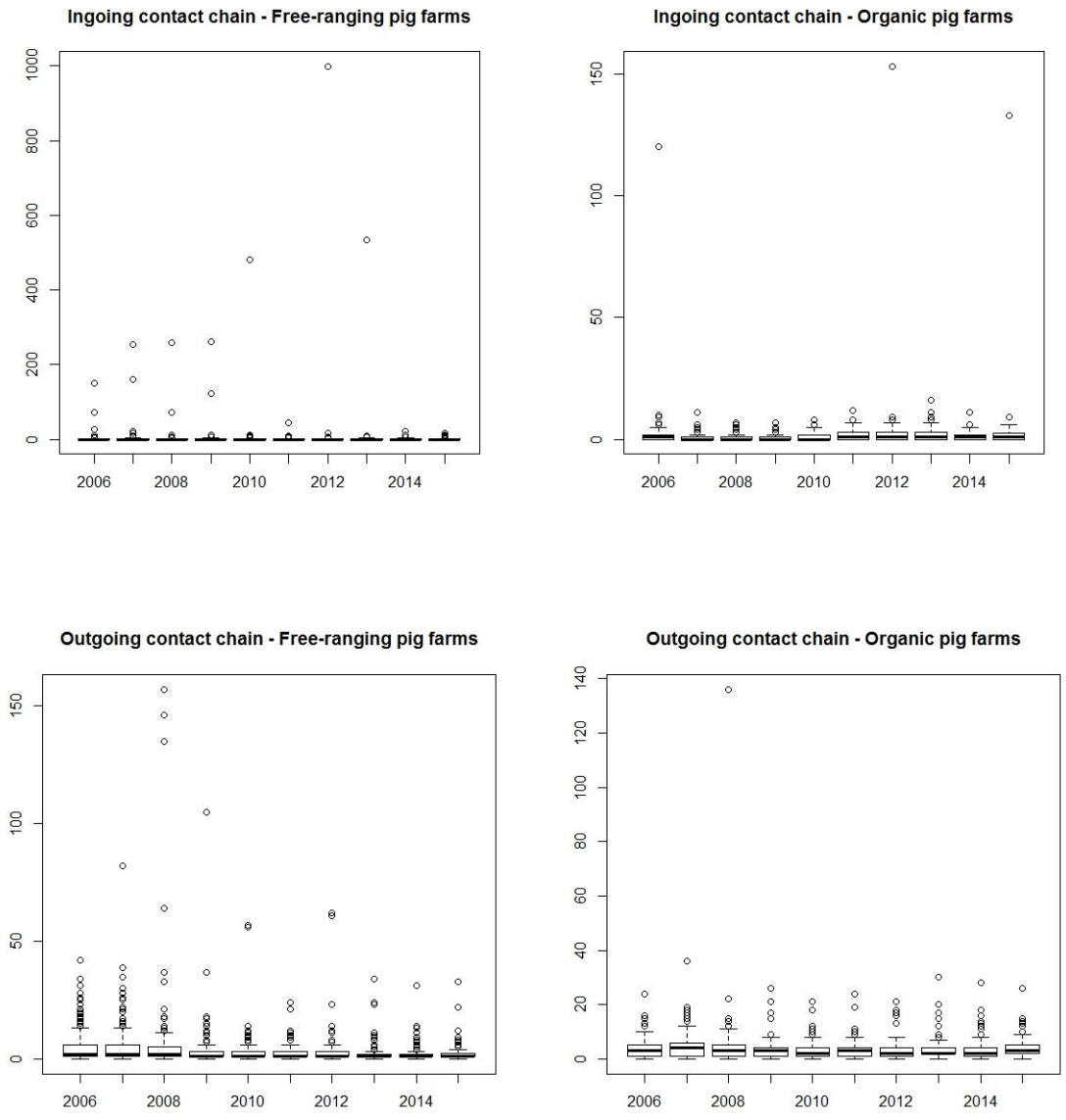


Figure 4. In-going and out-going contact chains for hobby sites.

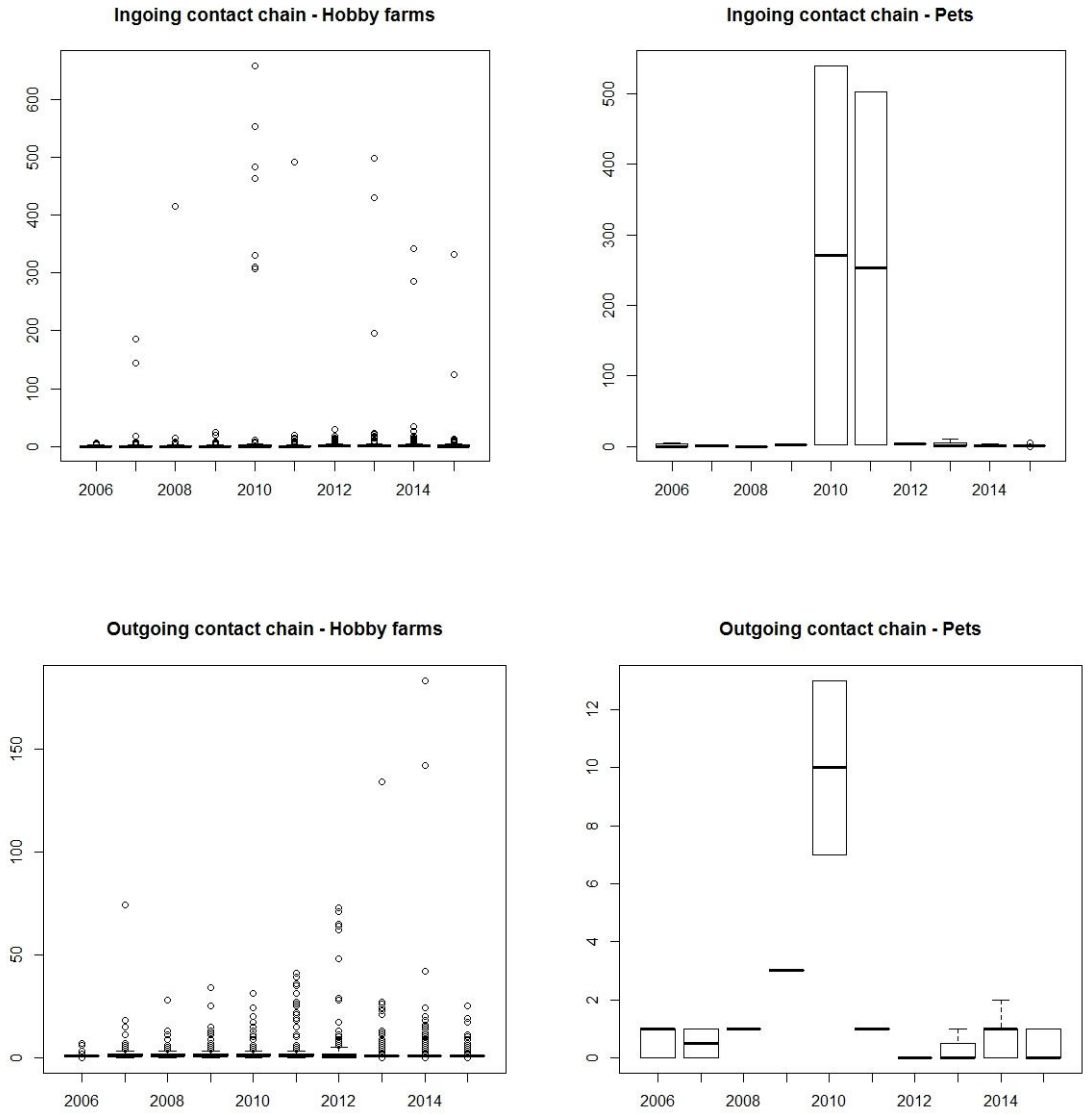


Figure 4 (continued). In-going and out-going contact chains for hobby sites.

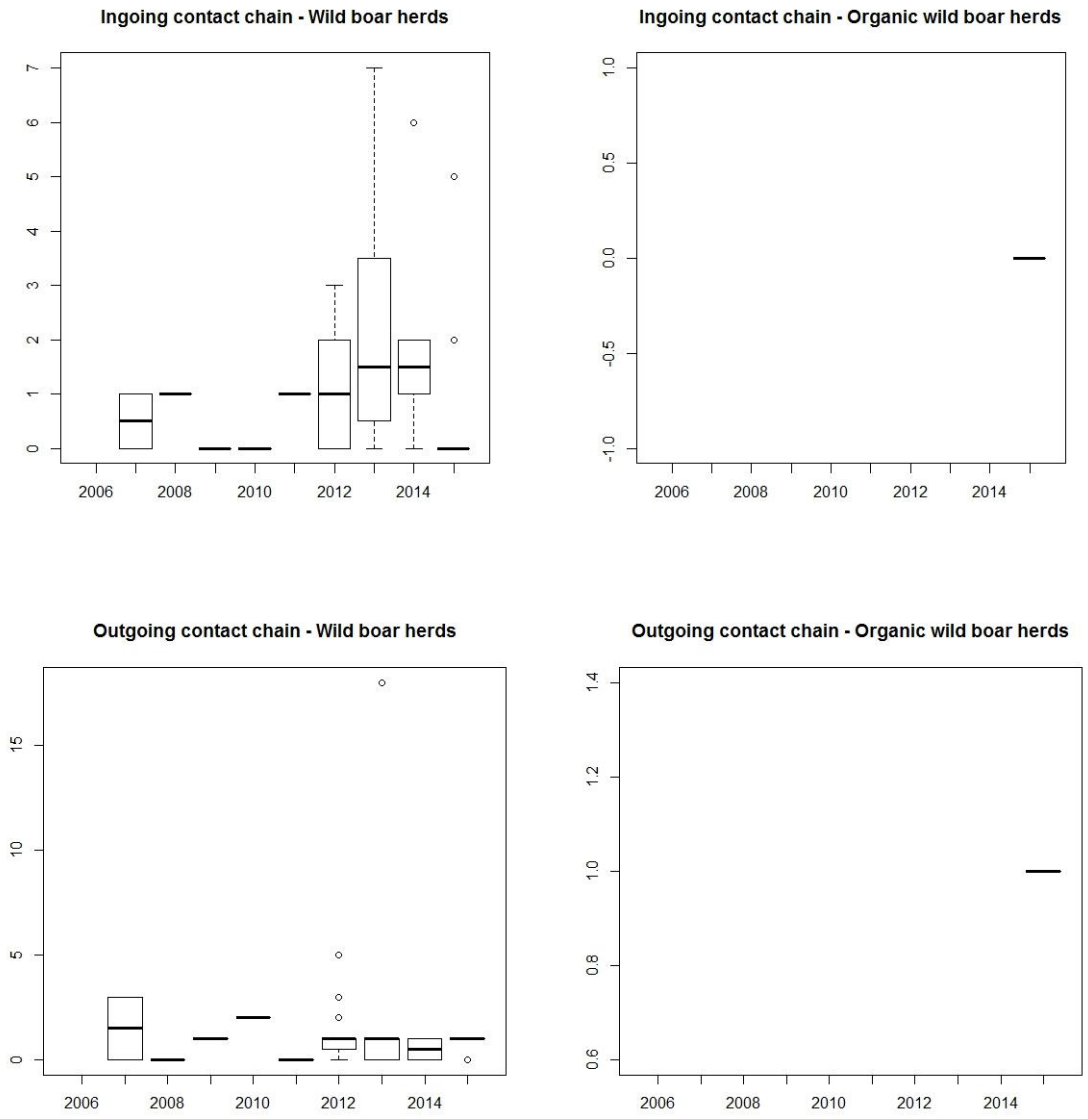
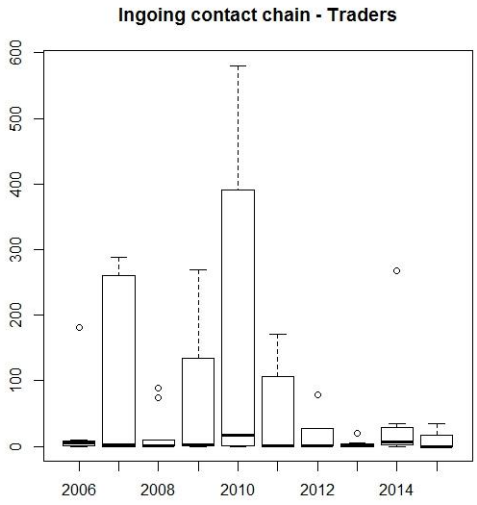
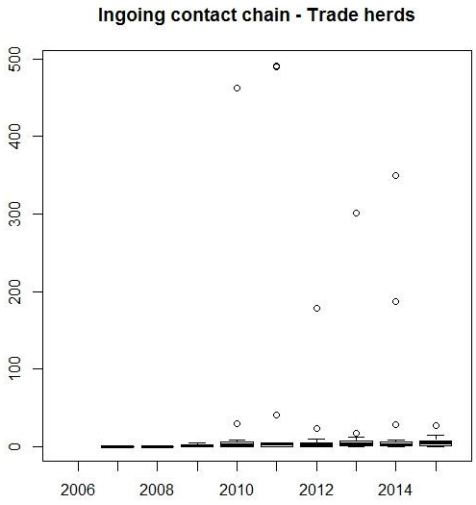
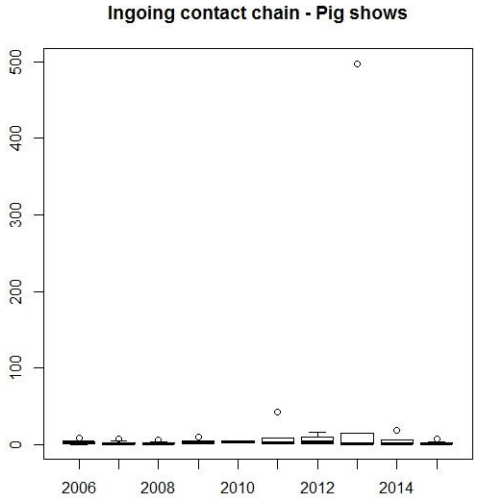
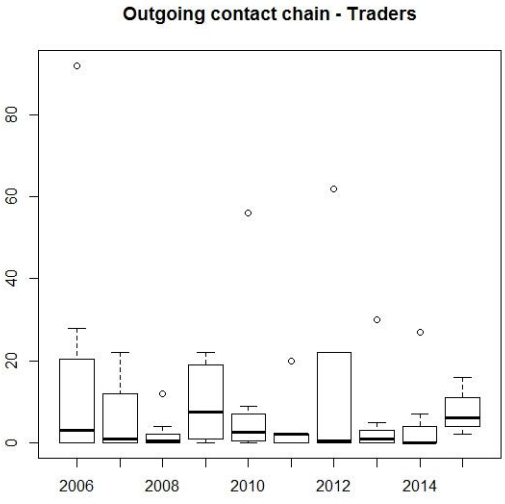


Figure 5. In-going and out-going contact chains for transit sites.


Ingoing contact chain - Traders
0
100
200
300
400
500
600
2006
2008
2010
2012
2014


Ingoing contact chain - Trade herds
0
100
200
300
400
500
2006
2008
2010
2012
2014


Ingoing contact chain - Pig shows
0
100
200
300
400
500
2006
2008
2010
2012
2014


Outgoing contact chain - Traders
0
20
40
60
80
2006
2008
2010
2012
2014

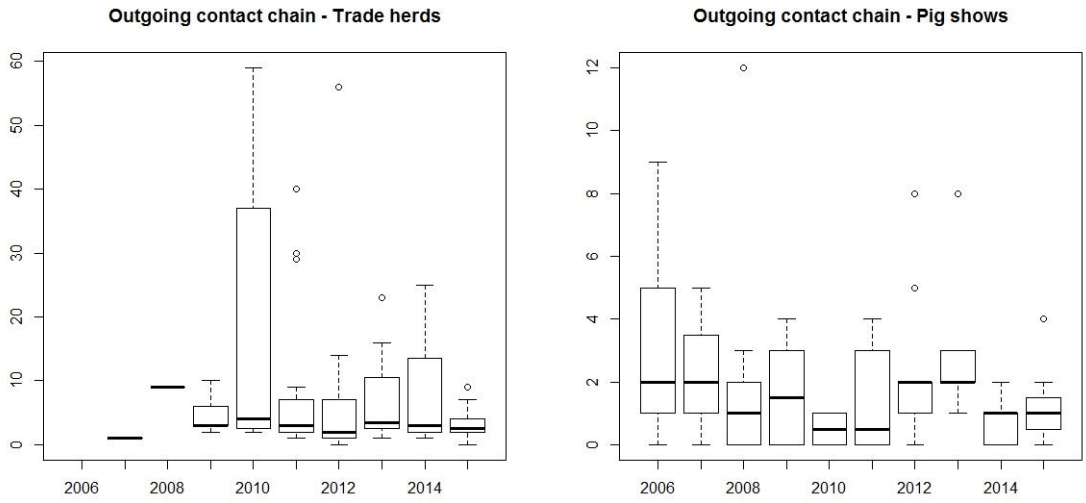
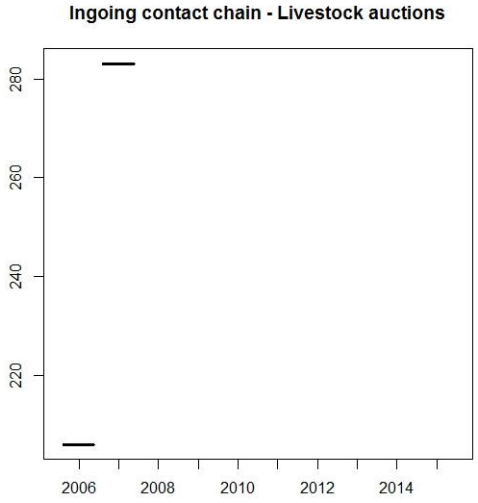
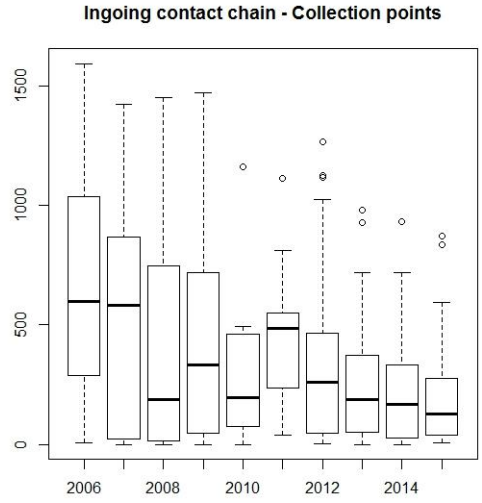
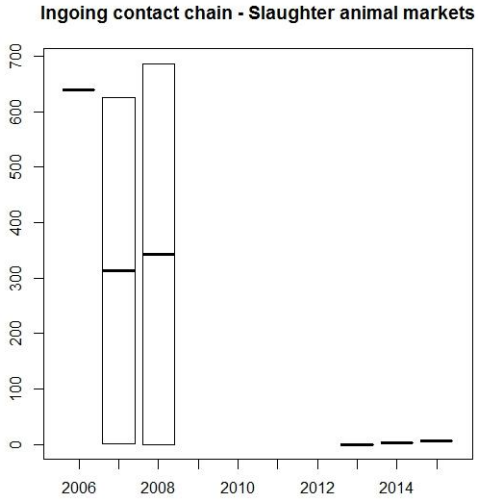
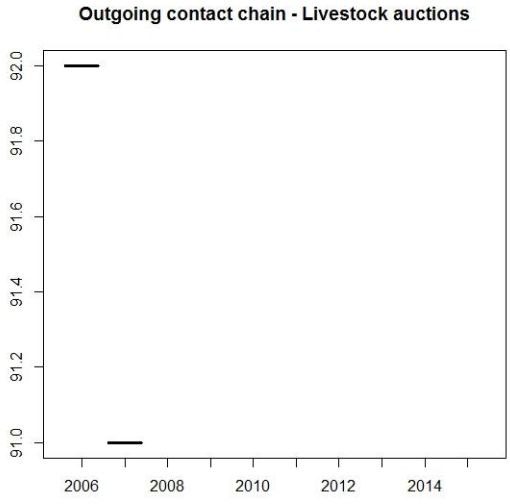


Figure 5 (continued). In-going and out-going contact chains for transit sites.


Ingoing contact chain - Livestock auctions
280
260
240
220
2006
2008
2010
2012
2014


Ingoing contact chain - Collection points
1500
1000
500
0
2006
2008
2010
2012
2014


Ingoing contact chain - Slaughter animal markets
700
600
500
400
300
200
100
0
2006
2008
2010
2012
2014


Outgoing contact chain - Livestock auctions
92.0
91.8
91.6
91.4
91.2
91.0
2006
2008
2010
2012
2014

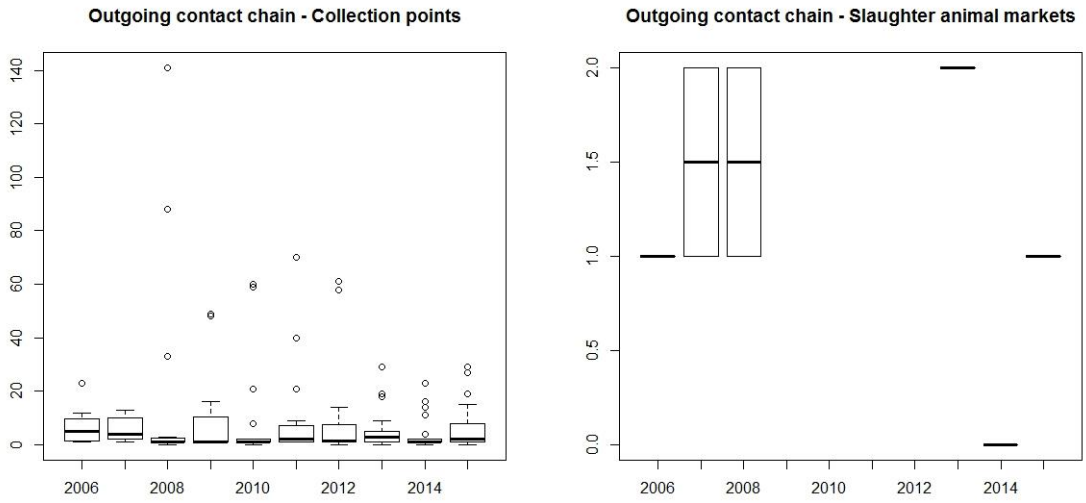


Figure 6. In-going and out-going contact chains for miscellaneous sites.

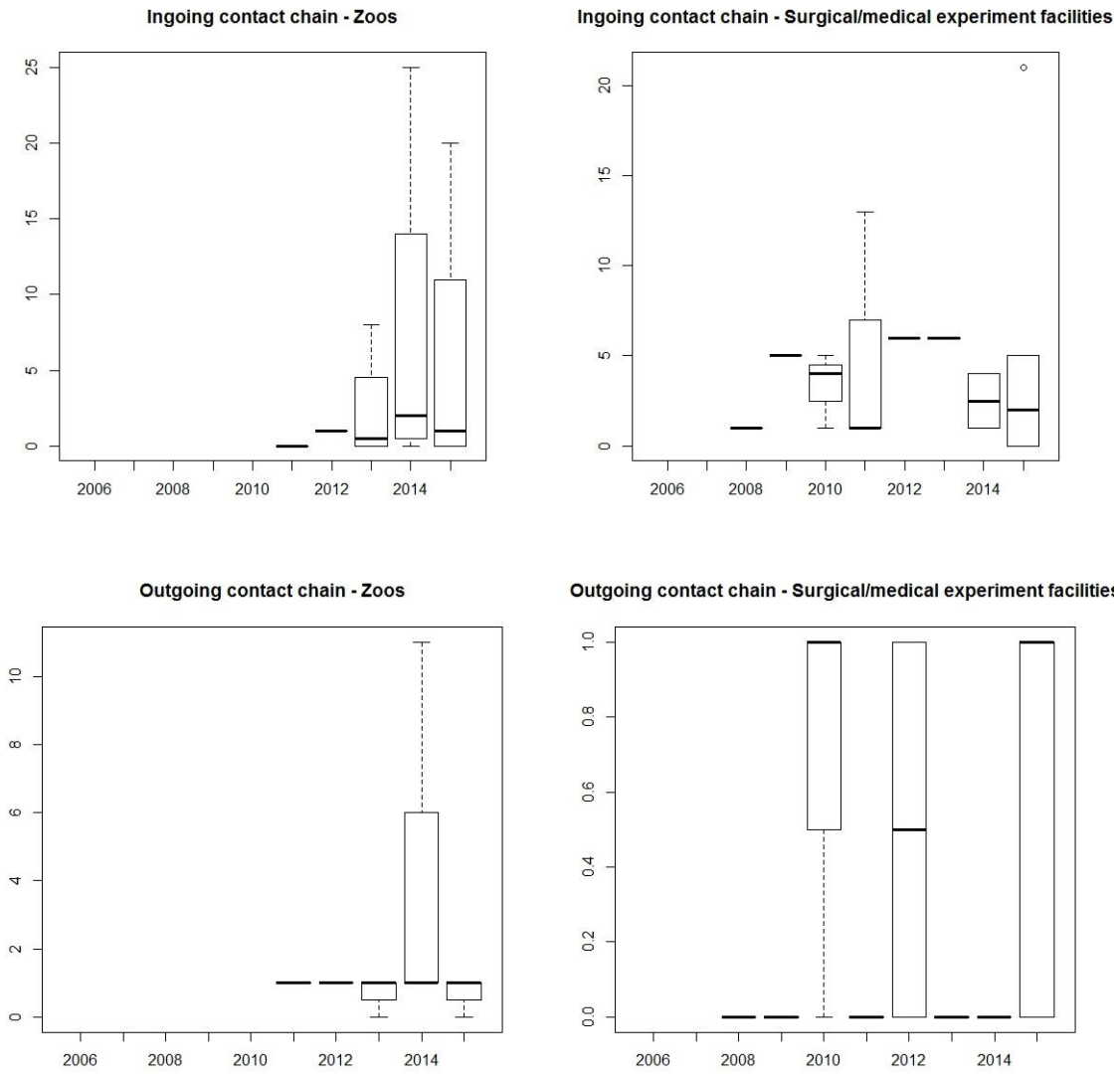
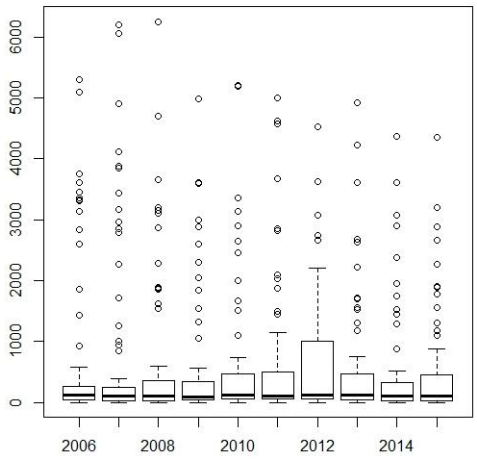
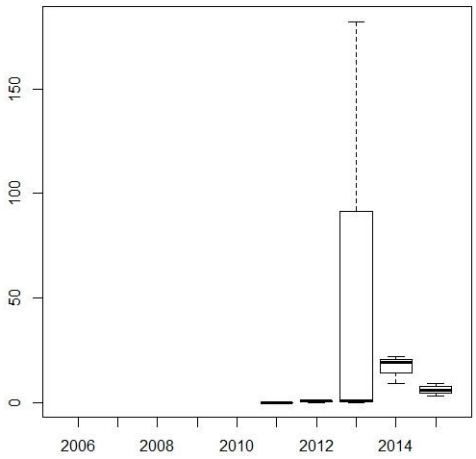


Figure 7. In-going and out-going contact chains for end of production sites.

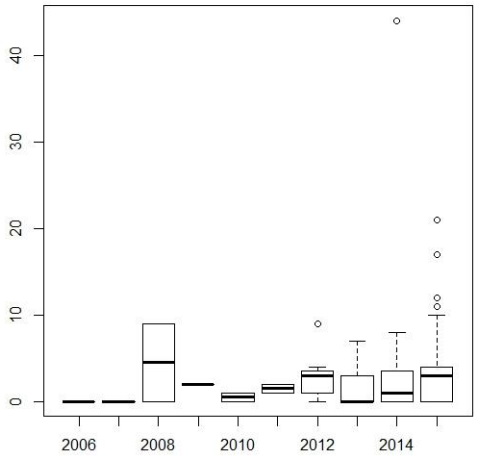
Ingoing contact chain - Slaughterhouses



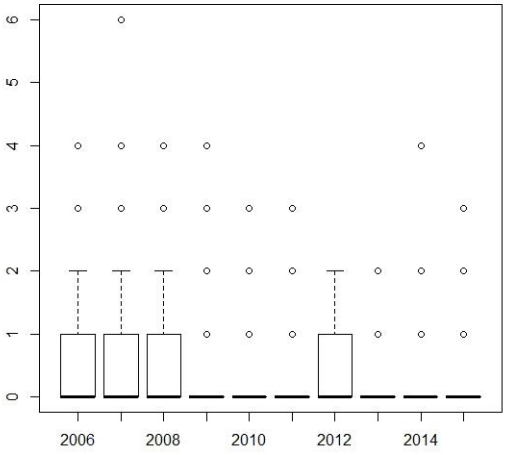
Ingoing contact chain - Export isolation facilities

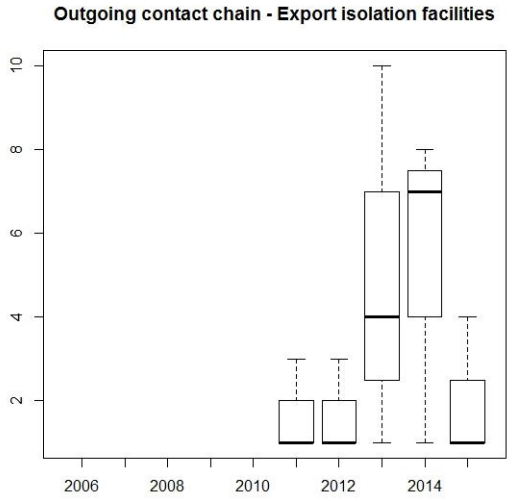


Ingoing contact chain - Collection points for dead animals



Outgoing contact chain - Slaughterhouses





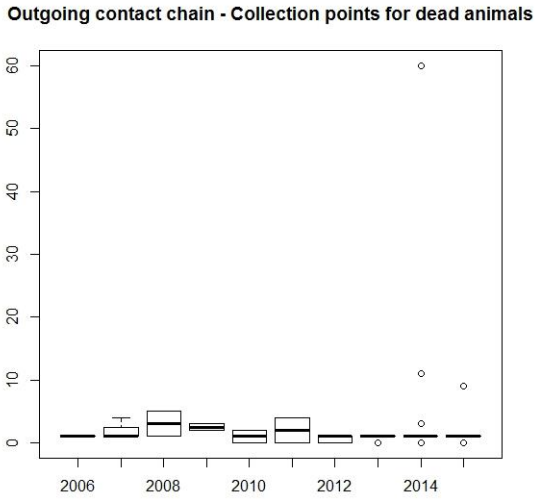
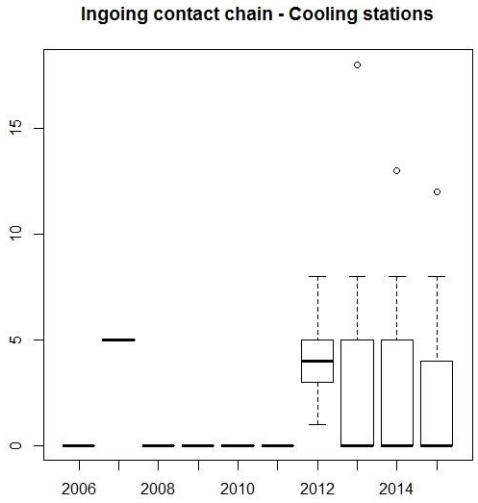
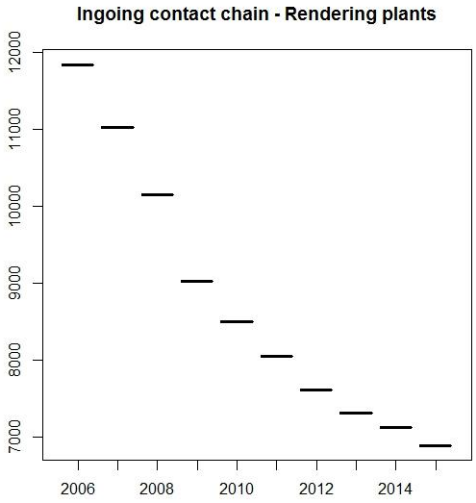
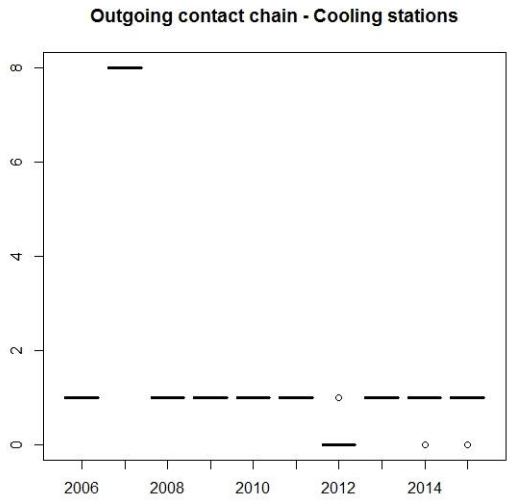
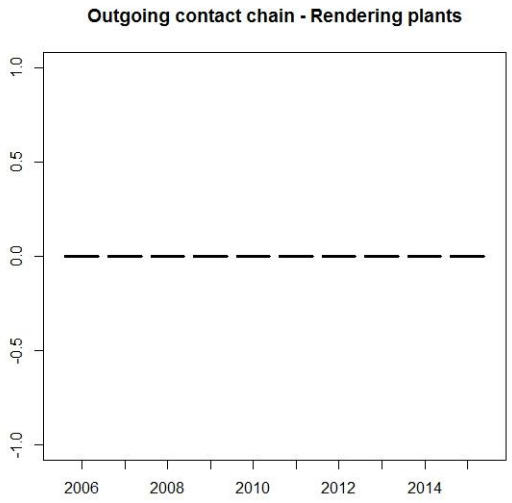


Figure 7 (continued). In-going and out-going contact chains for end of production sites.






Outgoing contact chain - Cooling stations
0
2
4
6
8
2006
2008
2010
2012
2014


Outgoing contact chain - Rendering plants
-1.0
-0.5
0.0
0.5
1.0
2006
2008
2010
2012
2014

9.2. Supplementary information for Manuscript II

9.2.1. Data preparation

Herd information. Holding information data included the holding and herd identification numbers. Several herds could be owned by the same farmer and therefore share an individual holding number, yet have different herd identification numbers. To keep the herds anonymous, new herd identification numbers were generated, and the herd was used as the unit of interest. Herds owned by the same farmer were marked to track the connection between them. The registered numbers of sows, weaners and finishers per year, as well as the herd type were extracted from the CHR.

The VetStat database records herd-level information on prescription-only drugs[1]. These data were used to add an antimicrobial usage index per year. The index was set to 1 if at least one prescription of tetracycline and/or beta-lactam was reported in the VetStat register.

In addition, the number of registered movements to abattoirs for each herd h per year was calculated based on the movement data and this value divided by 365, giving a herd-specific λ_a^h that was used as a parameter to model indirect contacts related to abattoir movements.

Our datasets lacked information on UTM coordinates for some of the herds. This information was updated using a website to calculate the coordinates based on the address of the holdings provided on the CHR website[2] (www.geoplaner.com/).

All herds were categorised according to: (1) herd type (breeding and multiplier herds, production herds, weaner herds, organic and free-range pig herds and hobby herds), (2) the proportion of sows, weaners and finishers registered in the CHR (Table S1), and (3) the type of production (Table S2).

No sows, weaners or finishers were registered in 181 herds in 2007 and 844 herds in 2014[3], but these herds had registered in-coming or out-going movements, and could therefore be considered active. The number of animals was estimated for these herds. For each herd type, the distribution of herds (with registered sows, weaners or finishers) was determined in each combination of categories 1-7 and A-C (Tables S1 - S2). In addition, the average number of sows, weaners and finishers registered in these herds in the CHR was calculated for each category combination. To estimate the missing number of animals, a category combination was randomly assigned to herds that had not registered any animals. Based on this combination, the number of sows, weaners and/or finishers was calculated using an exponential distribution with lambda given by the average number of sows, weaners and finishers.

Movement data. Information on the movements of swine was available from the movement database, including the holding and herd identification numbers for both sending and receiving herds, the date of the pig movements and the number of pigs moved. No information was available on the age group (sows, weaners or finishers) of pigs moved.

The age group of pigs moved out of the sending herd and into the receiving herd was estimated based on the herd categories 1-7 and A-C (Tables S1-S4). If no weaners were registered, but were thought to have been moved out of a herd, they were assumed to have been moved directly from the sow section.

9.2.2. MODELLING DISEASE SPREAD WITHIN A HERD

Environment-related recurrence. The probability decreases exponentially over time as follows:

$$f^{envir}(\Delta t) = \exp(-\alpha_{envir} \cdot \Delta t), \quad (1)$$

where Δt is defined as the time difference between the current simulation day and the day when LA-MRSA died out in the respective herd.

Within-herd dynamics. For each individual susceptible animal, the probability of infection $Prob_{C_i}^t$ at each time step t resulting from positive animals within compartment C_i was assumed to be density-dependent and was calculated based on the number of LA-MRSA-positive pigs $I_{C_i}^t$ and the total number of pigs $N_{C_i}^t$ within the individual compartment as follows:

$$Prob_{C_i}^t = 1 - \exp\left(-\frac{\beta_{C_i}^t * I_{C_i}^t}{N_{C_i}^t}\right), \quad (2)$$

where $\beta_{C_i}^t$ is the daily transmission rate of the infection for each compartment C_i, and

$i \in \{sows, weaners, finishers\}$. The probability of infection $Prob_{C_iC_j}^t$ resulting from positive animals from another compartment $C_j \neq C_i$ was calculated as:

$$Prob_{C_iC_j}^t = 1 - \exp\left(-\frac{\beta_{C_iC_j}^t * I_{C_j}^t}{N_{C_j}^t}\right), \quad (3)$$

where $\beta_{C_iC_j}^t$ is the between-compartment transmission rate. The total probability for each individual in compartment C_i was therefore:

$$Prob_{C_i}^t = 1 - (1 - Prob_{C_i}^t) * \prod_{j \neq i} (1 - Prob_{C_i C_j}^t). \quad (4)$$

In order to slow the within-herd spread, a time-dependent scaling function was introduced:

$$f(\Delta t) = 1 + exp\big(-k * (\Delta t - x)\big), \quad (5)$$

where Δt is the simulation time since the introduction of LA-MRSA in the herd, and k and x are read-in parameters defining the steepness and the midpoint of the scaling function. The within-compartment transmission rates $\beta_{C_i}^t$ were divided by the scaling function. The time-shifted transmission rates led to a similar but delayed level of within-herd prevalence (Figures S1-S5).

Mimicking the within-herd dynamics in 100 large production herds for 365 days using the transmission rates adapted by Broens et al. (2012)[4] led to a median within-herd prevalence of 65% for herds using high-risk antibiotics and 59% for herds that did not use high-risk antibiotics (Figures S1-S4).

Spread via indirect contacts. The transmission between two herds $\mathrm{H}_1$and H_2 with the same owner was modelled using equation (2) to calculate the probability of infection $Prob_{H_i H_j}^t$ with $C_i = H_2$ and $C_j = H_1$. The number of LA-MRSA-positive pigs $N_{H_1}^t$ ($I_{H_1}^t$) was equivalent to the total number of LA-MRSA-positive sows, weaners and finishers in H_1. If H_1 was a large herd, the low-risk between-compartment transmission rate of H_1 was used, whereas if H_1 was small, the within-herd transmission rate of H_1 was used. If transmission was successful, new LA-MRSA-positive pigs were introduced to the sow compartment of herd H_2.

9.2.3. FIGURES

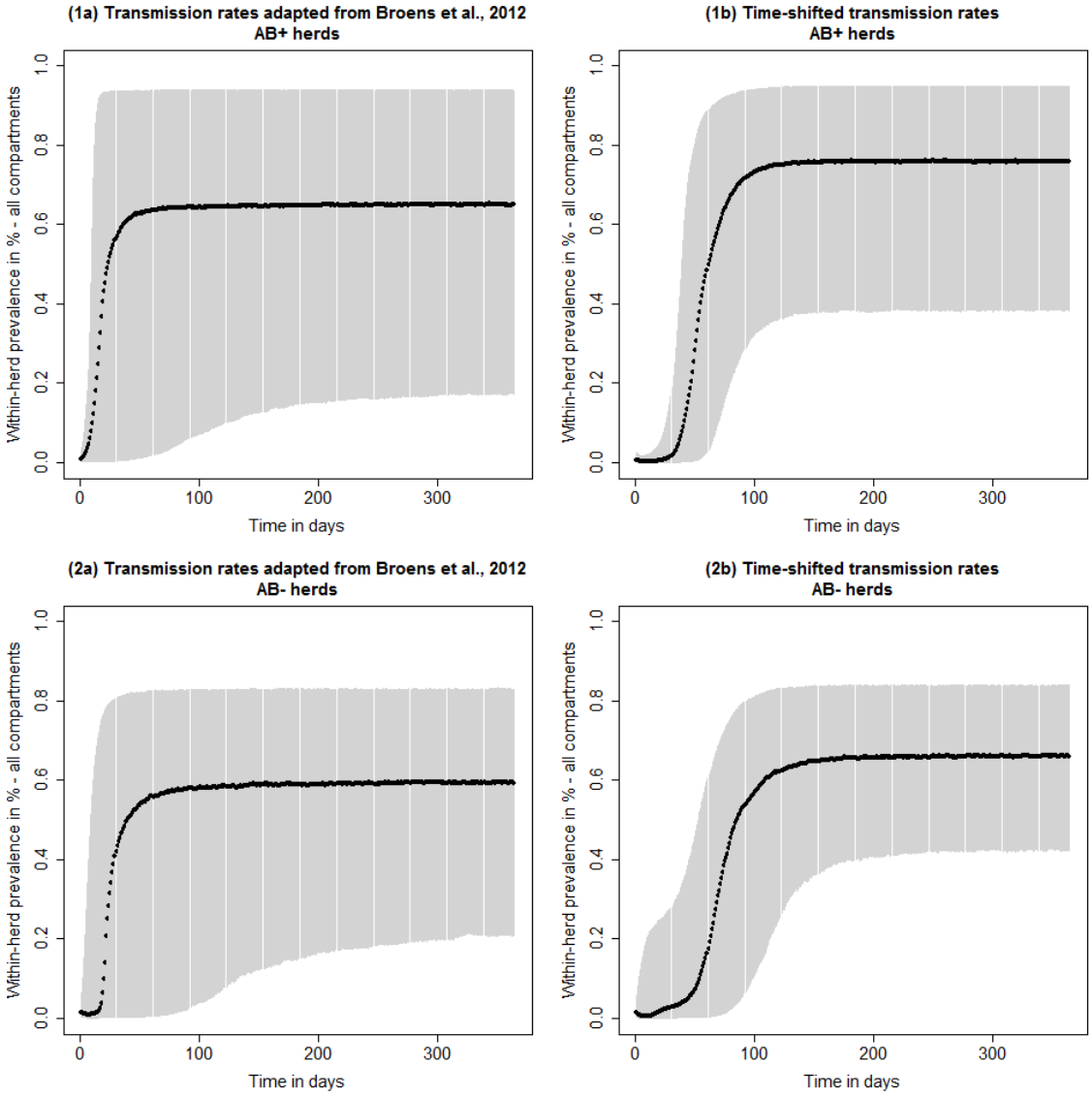


Figure S1. Development of the within-herd prevalence in large production herds in the first year after LA-MRSA initialisation in 100 production herds (1) using high-risk antibiotics, and (2) not using high-risk antibiotics for (a) transmission rates adapted from Broens et al.[4] (Supplementary Information, Table S5) and (b) time-shifted transmission rates. The black dotted line represents the median of 500 iterations and the grey area spans the 95% confidence interval.

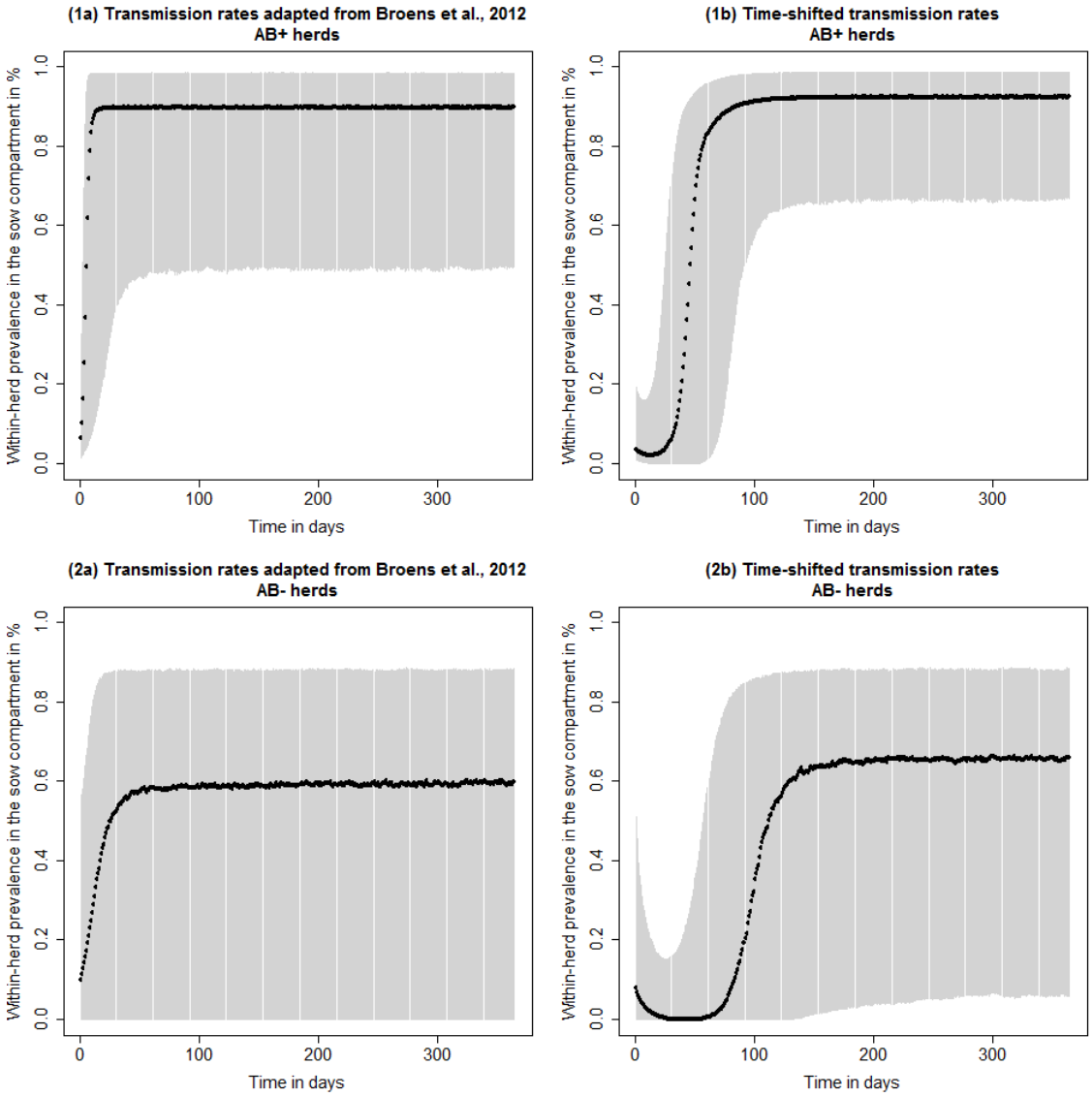


Figure S2. Development of the within-herd prevalence in the sow compartment in the first year after LA-MRSA initialisation in 100 production herds (1) using high-risk antibiotics, and (2) not using high-risk antibiotics for (a) transmission rates adapted from Broens et al.[4] (Supplementary Information, Table S5) and (b) time-shifted transmission rates. The black line represents the median of 500 iterations and the grey area spans the 95% confidence interval.

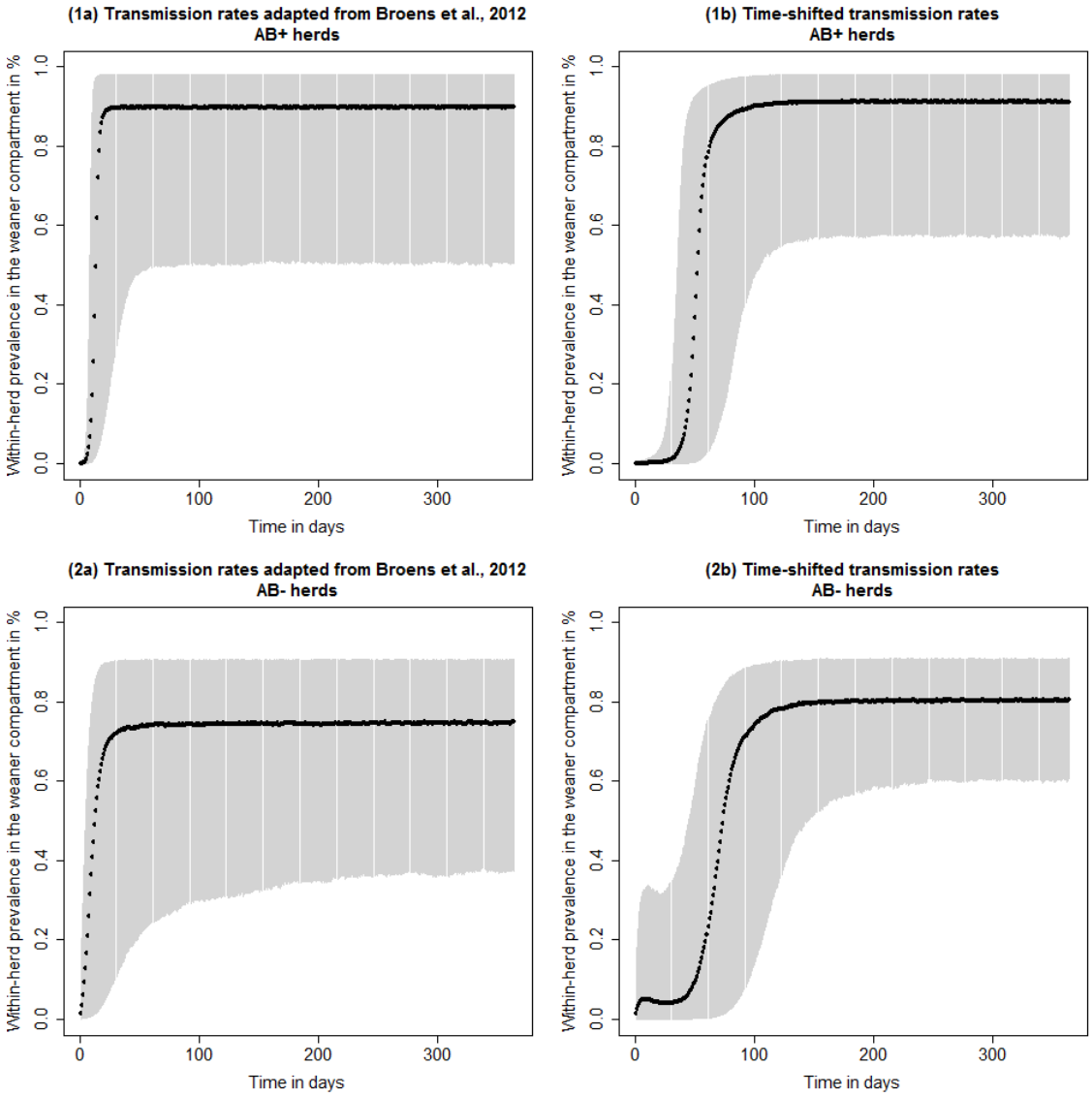


Figure S3. Development of the within-herd prevalence in the weaner compartment in the first year after LA-MRSA initialisation in 100 production herds (1) using high-risk antibiotics, and (2) not using high-risk antibiotics for (a) transmission rates adapted from Broens et al.[4] (Supplementary Information, Table S5), (b) time-shifted transmission rates and (3) time-shifted and scaled transmission rates. The black line represents the median of 500 iterations and the grey area spans the 95% confidence interval.

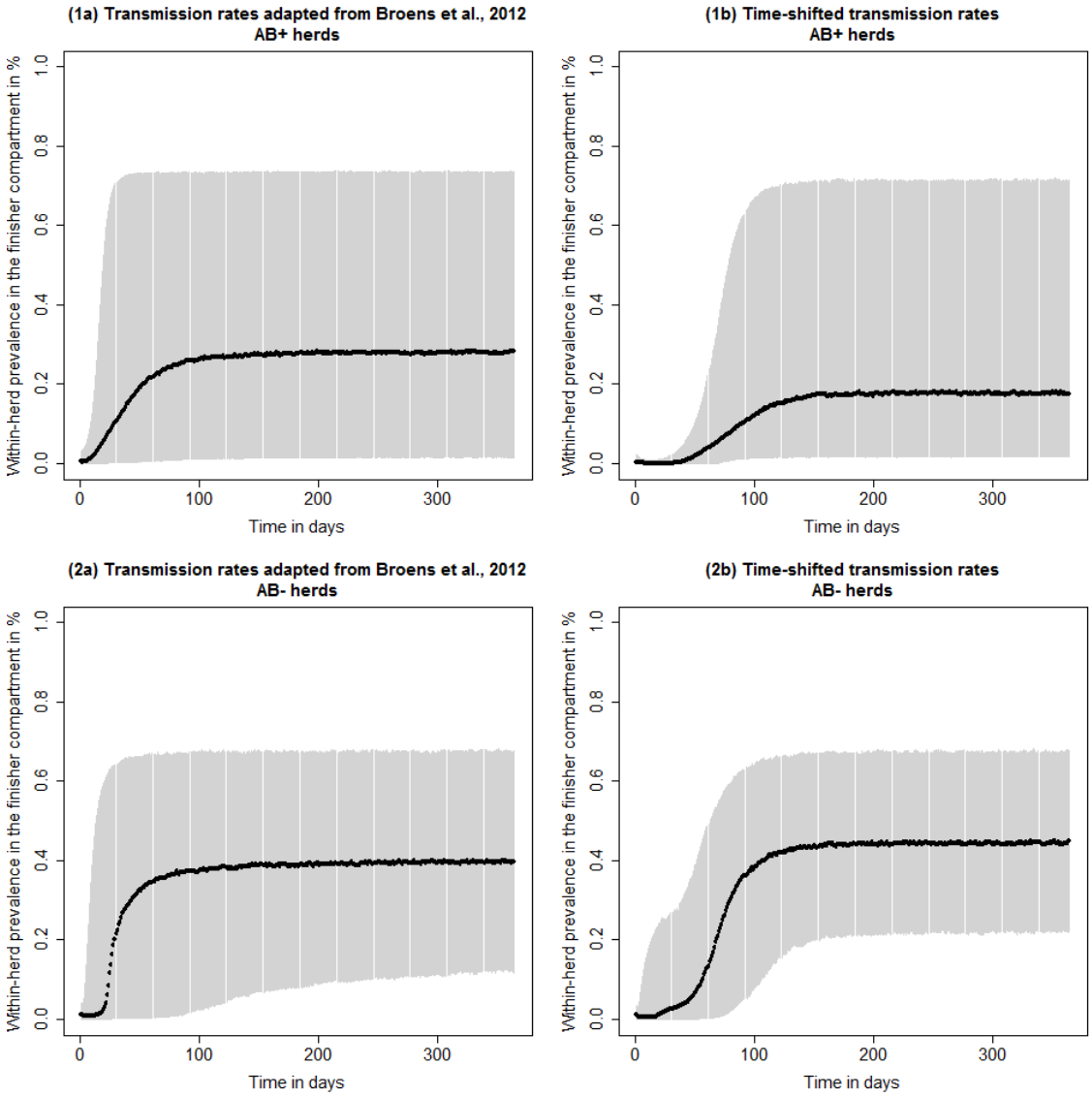


Figure S4. Development of the within-herd prevalence in the finisher compartment in the first year after LA-MRSA initialisation in 100 production herds (1) using high-risk antibiotics, and (2) not using high-risk antibiotics for (a) transmission rates adapted from Broens et al.[4] (Supplementary Information, Table S5) and (b) time-shifted transmission rates. The black line represents the median of 500 iterations and the grey area spans the 95% confidence interval.

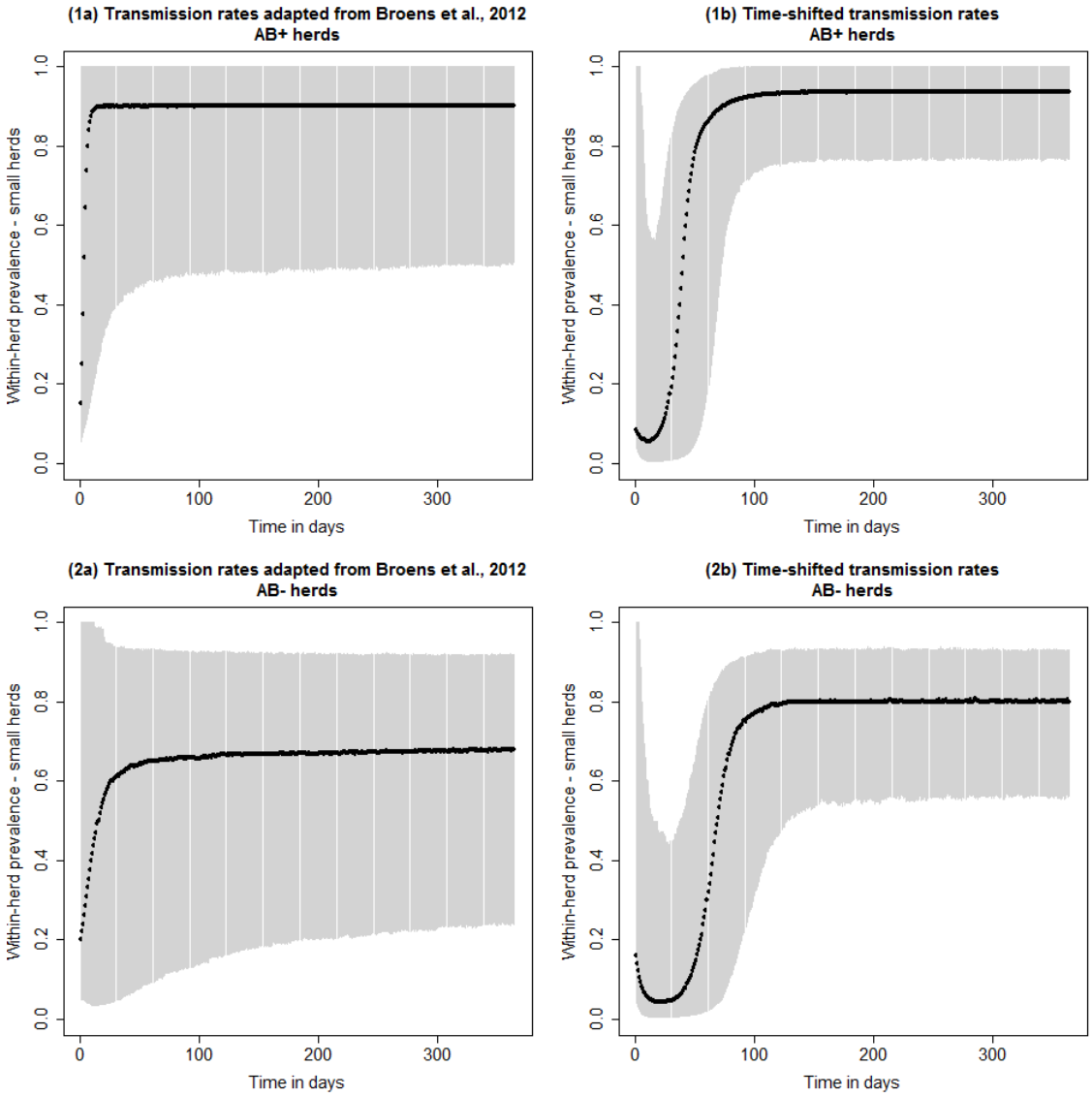


Figure S5. Development of the within-herd prevalence in small herds in the first year after LA-MRSA initialisation in 100 production herds (1) using high-risk antibiotics, and (2) not using high-risk antibiotics for (a) transmission rates adapted from Broens et al.[4] (Supplementary Information, Table S5) and (b) time-shifted transmission rates. The black line represents the median of 500 iterations and the grey area spans the 95% confidence interval.

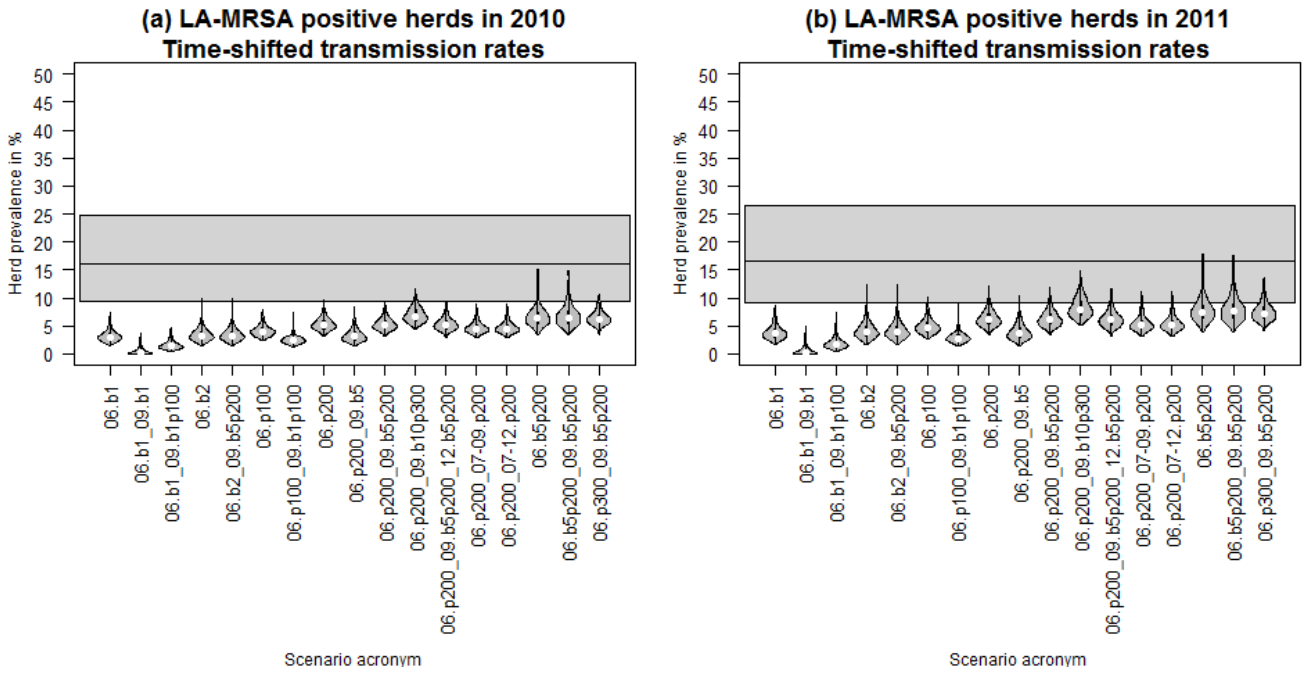


Figure S6. Violin plots summarising the herd prevalence in all herds in 2010 and 2011 based on 500 iterations of 17 LA-MRSA initialisation scenarios using time-shifted transmission rates adapted from Broens et al.[4] and modelling transmission only via animal movements.

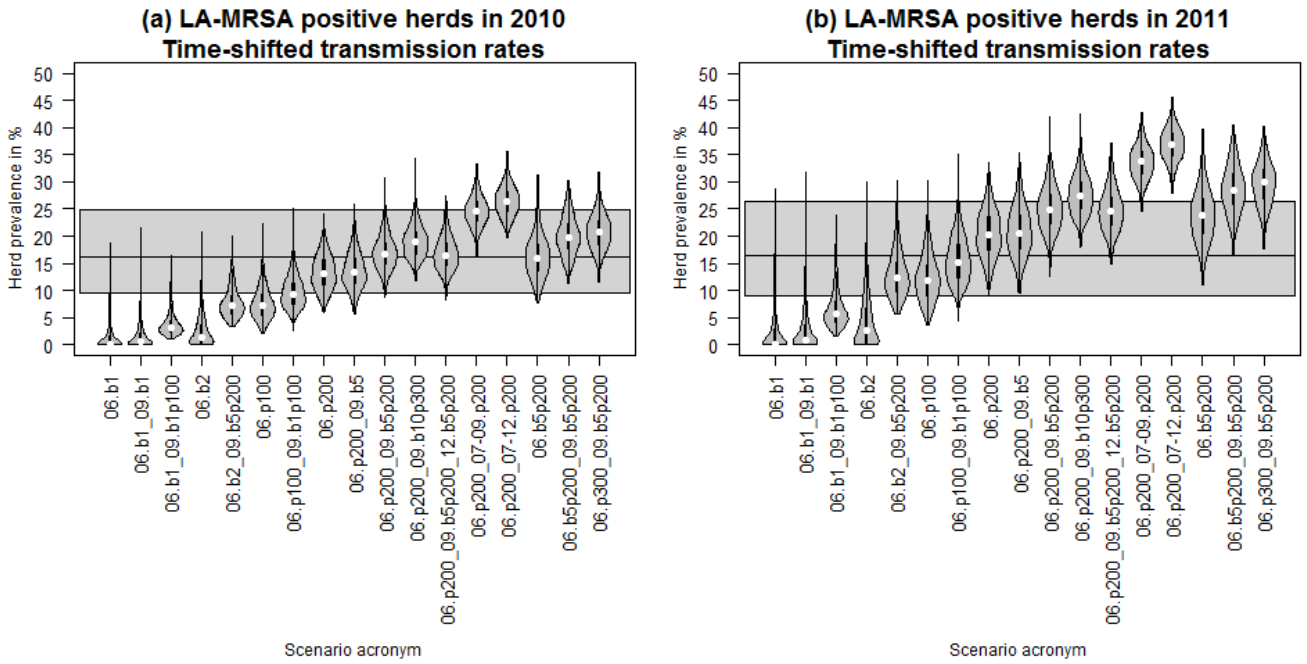


Figure S7. Violin plots summarising the herd prevalence in all herds in 2010 and 2011 based on 500 iterations of 17 LA-MRSA initialisation scenarios using time-shifted transmission rates adapted from Broens et al.[4] and modelling transmission via animal movements and via indirect contacts between herds.

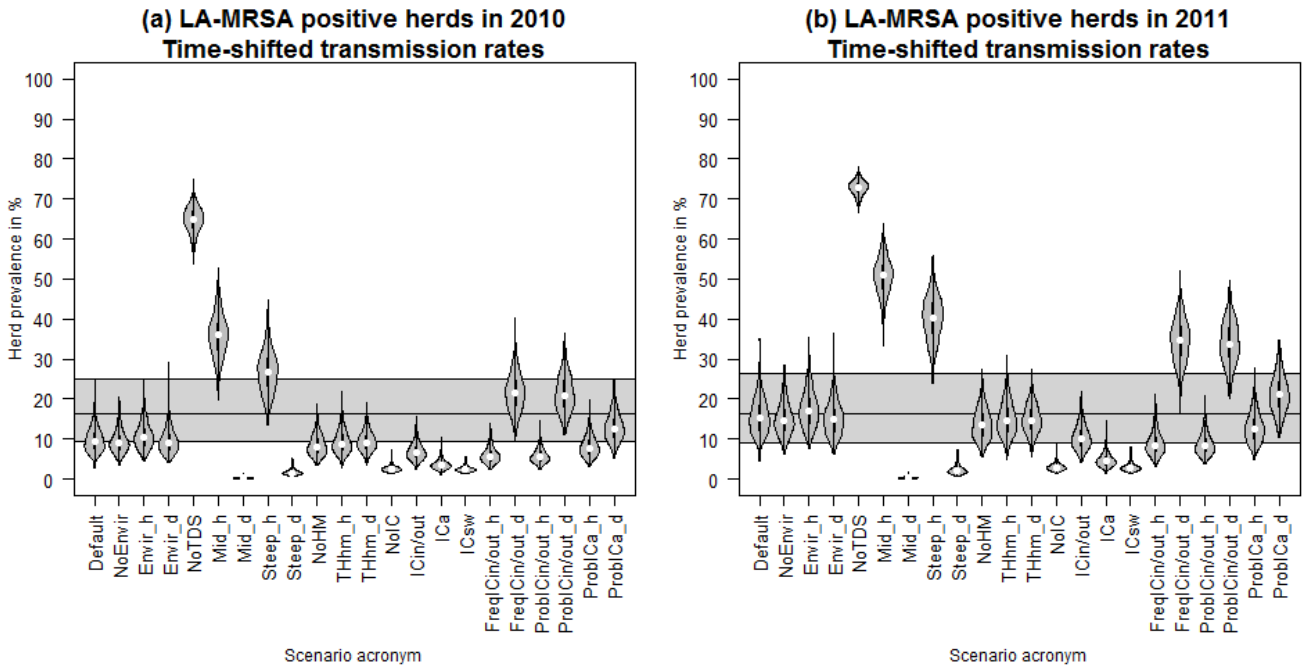


Figure S8. Violin plots summarising the herd prevalence in all herds in 2010 and 2011 based on 500 iterations of 21 LA-MRSA sensitivity analysis scenarios using time-shifted transmission rates adapted from Broens et al.[4], and modelling transmission via animal movements and via indirect contact among herds. The first violin represents the results of the default initialisation scenario 06.b1_09.b1p100.

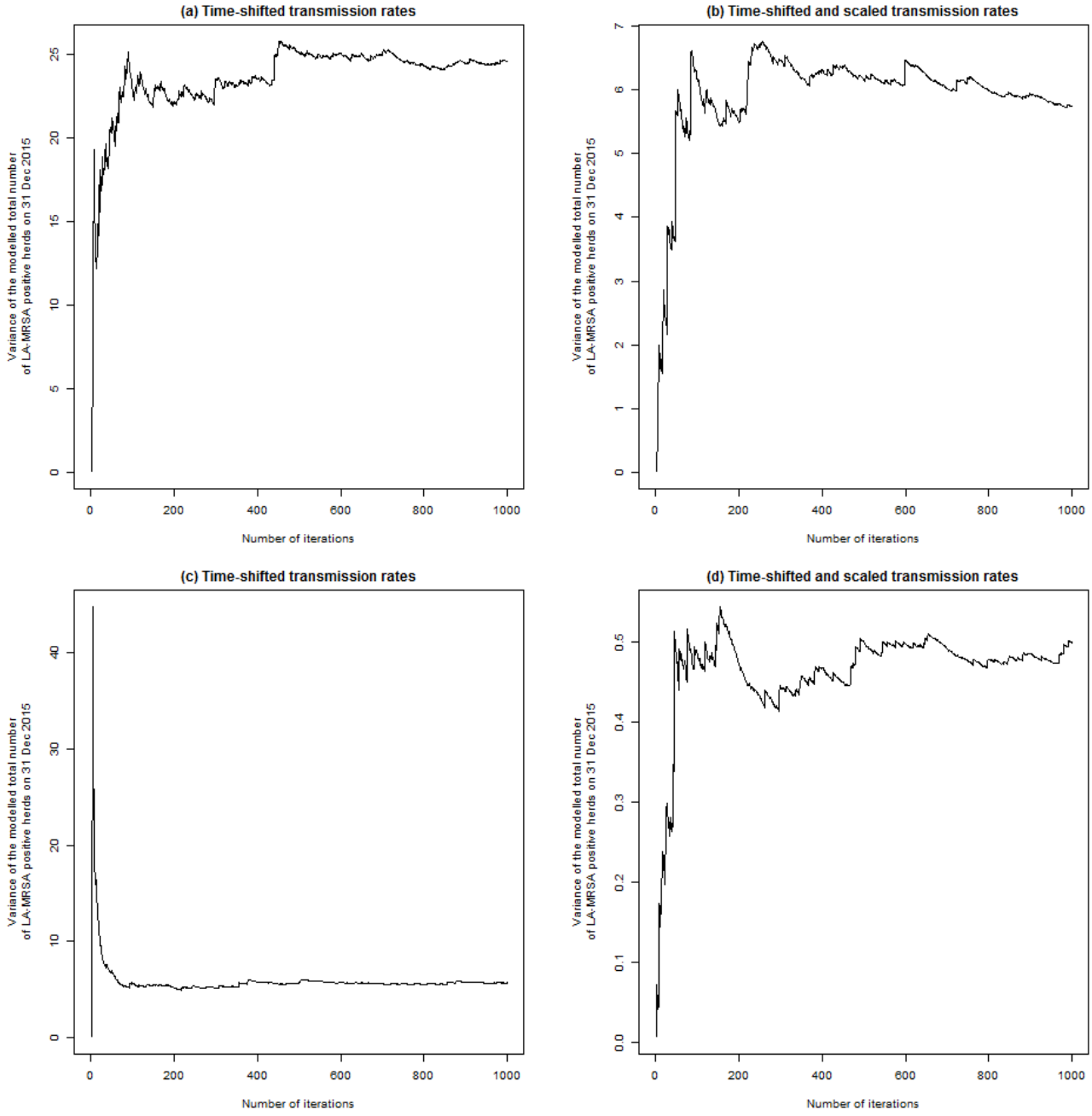


Figure S9. Variance of the total number of LA-MRSA-positive herds on 31st December 2015 against the number of simulation repetitions. Convergence is assumed when the variance stabilises. All convergence plots refer to the scenario with LA-MRSA initialisation in 100 production herds in 2006 and re-introduction for one breeding and multiplier herd and 100 production herds in 2009. (a) and (b) represent scenarios including indirect transmission routes, while (c) and (d) represent the results for transmission via pig movements only.

9.2.4. TABLES

Table S1. Herd categories related to the combination of sows, weaners, and finishers registered in the CHR, used to estimate the number of sows, weaners and finishers in herds that did not register any pigs.

Category	Sows registered	Weaners registered	Finishers registered
1	yes	yes	yes
2	yes	yes	no
3	yes	no	yes
4	no	yes	yes
5	yes	no	no
6	no	yes	no
7	no	no	yes

Table S2. Herd categories related to the type of production based on the registered number of sows and finishers.

Category	Type of production	Definition
A	Finisher herd	> 7.5 finishers per sow
B	Sow herd	< 5 finishers per sow
C	Integrated herd	5 – 7.5 finishers per sow

Table S3. Assumed age group (s = sows, w = weaners, f = finishers) of pigs moved out of the sending herd / in to the receiving herd. The letter x is used when the movement type is dependent on a defined threshold (Table S4). Below or equal to this threshold, finishers are assumed to be moved out of the sending herd and sows are assumed to be moved in to the receiving herd. Above this threshold, it is assumed that weaners/finishers were moved in to / out of the sending / receiving herd.

	Herd category of receiving herd										
Herd category of sending herd	**1A**	**1B**	**1C**	**2A**	**2B**	**2C**	**3B**	**4A**	**5B**	**6A**	**7C**
1A	w/f	f/s	f/s	w/f	f/s	f/s	f/s	w/f	f/s	w/f	w/w
1B	f/s	f/s	f/s	f/s	f/s	f/s	f/s	w/f	f/s	w/f	w/w
1C	x	x	x	x	x	x	f/s	w/f	f/s	w/f	w/w
2A	w/f	f/s	f/s	w/f	f/s	f/s	f/s	w/f	f/s	w/f	w/w
2B	f/s	f/s	f/s	f/s	f/s	f/s	f/s	w/f	f/s	w/f	w/w
2C	x	x	x	x	x	x	f/s	w/f	f/s	w/f	w/w
3B	w/f	w/f	w/f	w/f	w/f	w/f	w/s	w/f	w/s	w/f	w/w
4A	w/f	w/f	w/f	w/f	w/f	w/f	w/s	w/f	w/s	w/f	w/w
5B	w/f	w/f	w/f	w/f	w/f	w/f	w/s	w/f	w/s	w/f	w/w
6A	f/f	f/f	f/f	f/f	f/f	f/f	f/s	f/f	f/s	f/f	f/w
7C	w/f	w/f	w/f	w/f	w/f	w/f	w/s	w/f	w/s	w/f	w/w

Table S4. Assumed thresholds for setting the age group of movements if the movement type is given as *x* in Table S3. The thresholds are dependent on the registered herd type and for production, organic and hobby herds on the size of the herd. In small herds, homogeneous mixing of pigs is assumed, whereas separate compartments for the three age groups (sows, weaners, and finishers) were modelled for large herds. The number of pigs assumed to constitute a small herd was set to 200 animals.

Threshold for movement types	Production (small)	Organic (small)	Breeding and multiplier	Weaner	Hobby (small)	Production (large)	Organic (large)	Hobby (large)
Breeding sites								
Breeding and multiplier herds	3	3	50	20	3	50	50	50
Production sites								
Production (small)	3	3	3	3	3	3	3	3
Production (large)	3	3	20	20	3	20	20	20
Organic (small)	3	3	3	3	3	3	3	3
Organic (large)	3	3	20	20	3	20	20	20
Weaner herds	3	3	3	3	3	3	3	3
Hobby sites								
Hobby herds (small)	3	3	3	3	3	3	3	3
Hobby herds (large)	3	3	20	20	3	20	20	20

Table S5. Assumed values for a PERT distribution to define herd-specific cure rates and transmission rates based on the use of high-risk antibiotics, adapted by Broens et al.[4] The most likely (mode) and assumed minimum and maximum values for the PERT distributions were calculated based on values for R_0 (and their 95% CI) resulting from multivariable analysis of Dutch data, with 10.3 days taken to be the duration of the infectious period[5]. PERT distributions were defined as transformation of the Beta distribution with minimum (min), maximum (max) and most likely value (mode) and a mean $\mu = \frac{\min+4\cdot\text{mode}+\max}{6}$.

Use of high-risk antibiotics	Cure rate (all herds)	Within-compartment transmission rate	Low-risk between-compartment transmission rate	High-risk between-compartment transmission rate	Transmission rate in small herds
no	min = 0.056, max = 0.385, mode = 0.097	min = 0.111 max = 0.856 mode = 0.307	min = 0.00175 max = 0.00301 mode = 0.00233	min = 0.07184 max = 0.48155 mode = 0.18301	min = 0.111 max = 0.856 mode = 0.307
yes		min = 0.211 max = 2.924 mode = 0.784	min = 0.00330 max = 0.01029 mode = 0.00583	min = 0.13689 max = 1.64515 mode = 0.46796	min = 0.211 max = 2.924 mode = 0.784

Table S6. Distance probabilities of indirect contact among herds for the different types of contact and distance categories.

Distance category (up to … km)	Contact from indoor herd	Contact from outdoor herd	Contact related to abattoir movements
1	0.12	0.12	0.0054
3	0.24	0.24	0.0224
10	0.30	0.29	0.1898
15	0.083	0.089	0.1503
20	0.083	0.089	0.1252
30	0.054	0.050	0.1747
40	0.01714	0.01714	0.1347
50	0.01714	0.01714	0.0823
60	0.01714	0.01714	0.0454
70	0.01714	0.01714	0.0274
80	0.01714	0.01714	0.0141
90	0.01714	0.01714	0.0099
100	0.01714	0.01714	0.0108
110	0	0	0.0041
120	0	0	0.0025
130	0	0	0.00018
140	0	0	0.00006
150	0	0	0.00006
160	0	0	0.00006
170	0	0	0.00006
180	0	0	0.00006
190	0	0	0.00006
200	0	0	0.00006
210	0	0	0.00006
220	0	0	0.00006
230	0	0	0.00006
240	0	0	0.00006
250	0	0	0.00006
350	0	0	0.00006
1,000	0	0	0.00006

Table S7. LA-MRSA initialisation scenarios tested using the developed simulation model.

Acronym	First introduction of LA-MRSA in the simulation model	Initialisation of LA-MRSA-positive herds at later points in time
06.b1		none
06.b1_09.b1	**2006:** - 1 breeding and multiplier herd	**2009:** - 1 breeding and multiplier herd
06.b1_09.b1p100		**2009:** - 1 breeding and multiplier herd - 100 production herds
06.b2		none
06.b2_09.b5p200	**2006:** - 2 breeding and multiplier herds	**2009:** - 5 breeding and multiplier herds - 200 production herds
06.p100		none
06.p100_09.b1p100	**2006:** - 1% of production herds (100 herds)	**2009:** - 1 breeding and multiplier herd - 100 production herds
06.p200		none
06.p200_09.b5		**2009:** - 5 breeding and multiplier herds
06.p200_09.b5p200		**2009:** - 5 breeding and multiplier herds - 200 production herds
06.p200_09.b10p300		**2009:** - 10 breeding and multiplier herds - 300 of production herds
06.p200_09.b5p200_12.b5p200	**2006:** - 2% of production herds (200 herds)	**2009:** - 5 breeding and multiplier herds - 200 production herds **2012:** - 5 breeding and multiplier herds - 200 production herds
06.p200_07-09.p200		**2007-2009:** - 200 production herds each year
06.p200_07-12.p200		**2007-2012:** - 200 production herds each year
06.b5p200	**2006:** - 2% of production herds (200 herds)	none
06.b5p200/09.b5p200	- 2% of breeding and multiplier herds (5 herds)	**2009:** - 5 breeding and multiplier herds - 200 production herds
06.p300/09.b5p200	**2006:** - 3% of production herds (300 herds)	**2009:** - 5 breeding and multiplier herds - 200 production herds

9.2.5. REFERENCES

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5. Broens, E. M., Graat, E. A. M., van de Giessen, A. W., Broekhuizen-Stins, M. J. & de Jong, M. C. M. Quantification of transmission of livestock-associated methicillin resistant Staphylococcus aureus in pigs. *Vet. Microbiol.* **155,** 381–388 (2012).